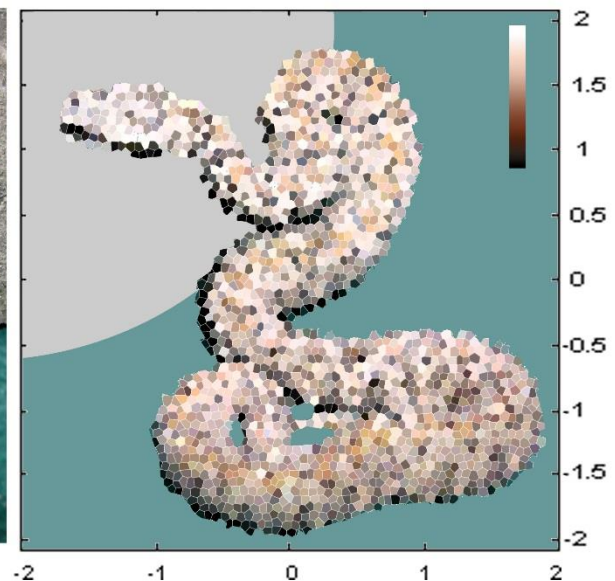




Biodiversity of the Snakes in Northern and Western Mountains of Iran, with Special Emphasis on Biodiversity in Colubroids



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Cover illustration: A conceptual inference from the ability of modern science to give a proper picture of the complexity within the living organisms.

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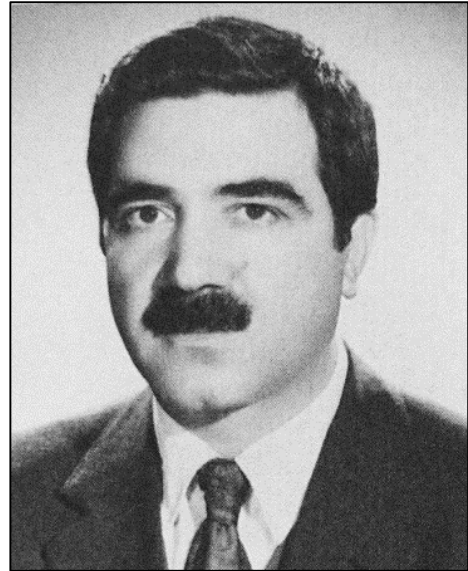
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I dedicate my dissertation to
Dr. Mahmoud Latifi
his great book, snakes of Iran, inspired me
to study about biodiversity of the snakes
in Iran



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CHAPTER 1

Introduction

1-1- General context

1-1-1- Evolution of snakes

Snakes are limbless (or nearly so) reptiles¹, with elongated bodies accomplished by an increase in number of vertebrae (typically 120 – 240 vertebrae including more than 30 presacral), each with a pair of free ribs in the neck and trunk, creating a very flexible body that permits extremely efficient undulatory locomotion in water, on and underground and in bushes and trees. The body of snakes is covered with epidermal scales. They are carnivores and without limbs, most of the snakes rely for feeding on their modified cranial anatomy, leading to the snakes extraordinary gape size and ability to consume big preys (Zug *et al.*, 2001). All of this enables snakes to successfully diversify and occupy very different terrestrial ecosystems (except polar) and even have successful a marine radiation (Pough *et al.*, 2003).

The evolution of snakes roots back to the lizards, but the exact identity of the snakes ancestors and sister groups in lizards is not well understood yet. Recent molecular data indicate that almost all groups of snakes arose from within a type of burrowing blind snakes, called scolecophidians (Wiens *et al.*, 2012). On the other hand, osteological data showed that mosasaurs, which are large, extinct marine varanoid lizards², represent a crucial intermediate stage between lizards and snakes. Also, some herpetologists argue that from a soft anatomy (i.e. tongue) point of view, snakes are most closely related to terrestrial varanoid lizards (Rieppel and Zaher, 2000).

¹ : The word "Reptile" in this thesis refer to the traditional class Reptilia, which is a paraphyletic group comprising all non-avian and non-mammalian amniotes

² : Mosasaur lineage diverged from that of monitors at least 100 mya (Pianka *et al.*, 2004).

1-1-2- Snake phylogeny and classification

The suborder Ophidia (Squamata, Sauropsida), with about 3432 identified species (see <http://www.reptiledatabase.org>) are the second most specios group of living reptiles. There are numerous debates on the systematics of snakes. Both molecular (Wiens *et al.*, 2012; Vidal *et al.*, 2007; Slowinski and Lawson, 2002; Vidal and Hedges, 2002; Heise *et al.*, 1995) and osteological, soft anatomical and ecological (Lee and Scanlon, 2002) data showed that snakes diverged early into the sister taxa scolecophidians and alethinophidians. Scolecophidians or blind snakes are burrowing snakes, whereas alethinophidians occupy a diversity of ecological niches. Blind snakes contain three major clades: Anomalepididae, Leptotyphlopidae and Typhlopidae (Figure 1-1). Leptotyphlopidae are distributed in the tropics and subtropics of Africa and the Americas, and Southwestern Asia. Typhlopidae are cosmopolitan in tropical regions but also is present in south Europe and southwestern Asia.

The branching pattern and sister-group relationship is less understood in alethinophidians. Skeletal and molecular data suggest an early divergence in the alethinophidians, with one lineage giving rise to the glossy snakes and the other to the remaining snakes. Among the remaining alethinophidia, the caenophidians (derived snakes, ca. 2470 sp.) widely use venom to subdue their prey, whereas the remaining alethinophidian snakes with about 170 species, referred to as henophidian snakes, use constriction (Vidal *et al.*, 2007). Henophidia don't form a monophyletic group and taxonomically are not stable yet. Following Vidal *et al.* (2007), Henophidia is composed of 12 lineages, including the family Boidae. Only the subfamily Erycinae of family Boidae is present in southwestern Asia.

The caenophidian lineage is considered as the *Acrochordus* snakes (family Acrochordidae) and the remaining advanced snakes are referred to as Colubroidea. *Acrochordus* is considered to be the sister group to the colubroids, with more than 2500 extant species thus including the majority of all snake species. Colubroidea is one of the most conspicuous and phylogenetically well-known radiations of terrestrial vertebrates. Crown-group Colubroidea is considered to consist of the extant families Colubridae, Elapidae, Homalopsidae, Lamprophiidae, Pareatidae, Viperidae, and Xenodermatidae (Pyron *et al.*, 2011) (Figure 1-2). Colubridae, Elapidae, and Viperidae are present in southwestern Asia.

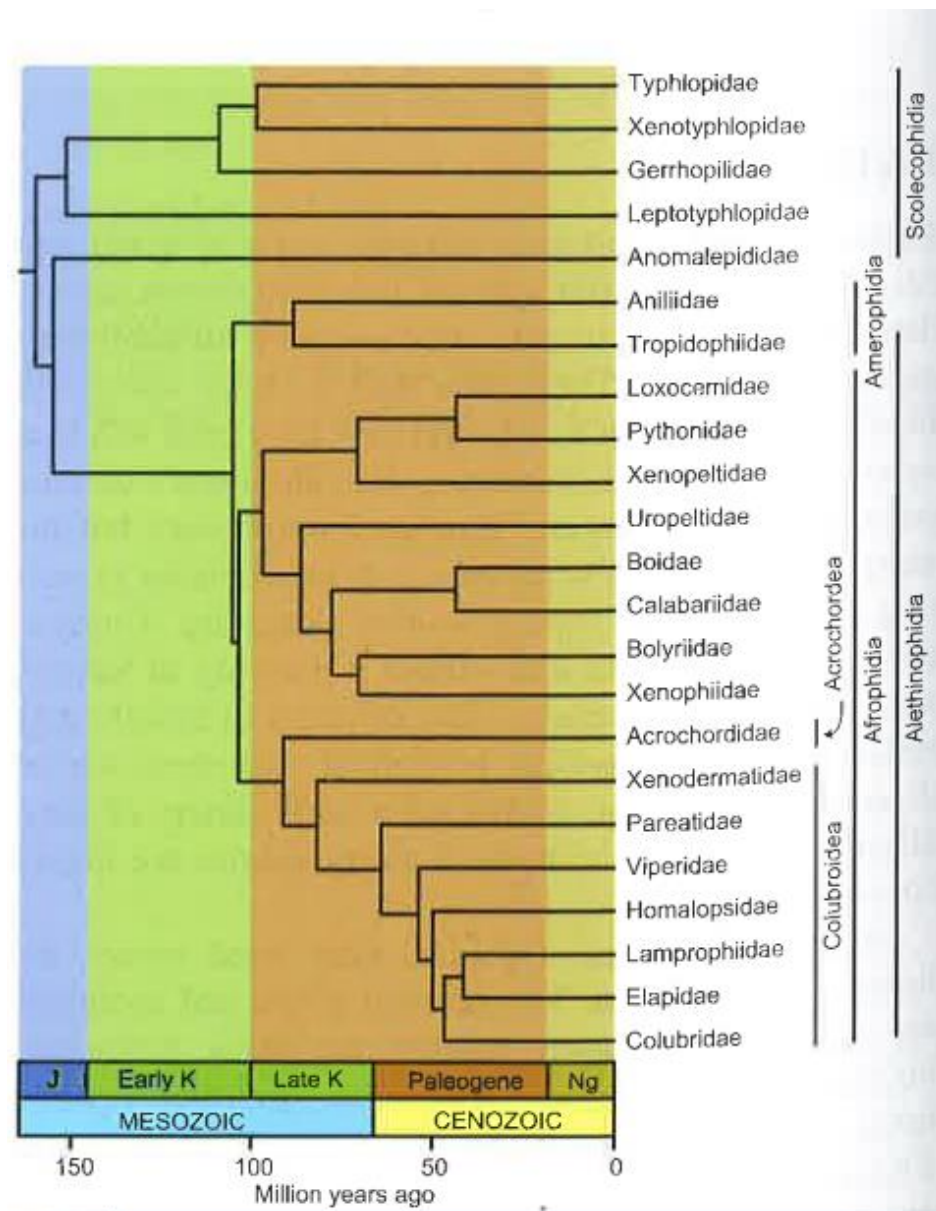


Figure 1-1. Time tree showing phylogenetic relationships among snake higher taxonomy (In Vitt and Caldwell, 2013, Based mostly on Vidal and Hedges, 2007; Vidal *et al.*, 2009).

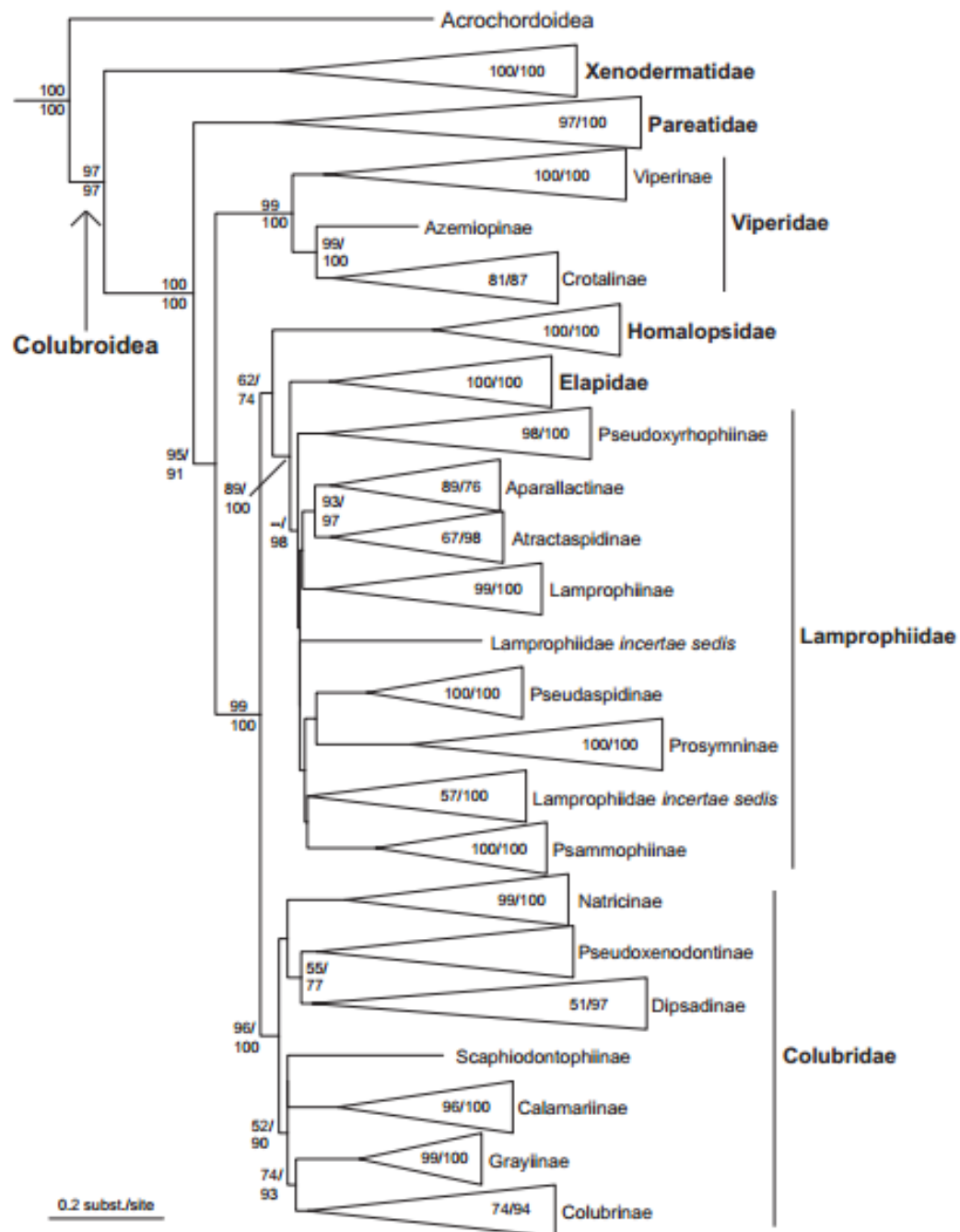


Figure 1-2. Phylogenetic relationship among colubroid snakes. The tree was estimated based on a likelihood analysis of five concatenated genes. Numbers next to branches or in terminal triangles indicate BS/SHL support values. (Pyron *et al.*, 2011)

1-1-3- Global matters about snake biodiversity

It is estimated that still more than 10% of reptile species (including snakes) have not been described yet (IUCN, 2009). Only from February 2012 to February 2013, 54 new species of

snakes were described around the world. Most of this advances are indebted to progress in developing molecular techniques (primarily DNA sequencing) that have transformed the ability of scientists to describe and define biological diversity (Bickford *et al.*, 2007). But on the other hand, recent studies show that globally 12% of snake species are estimated to be threatened with extinction (Böhm *et al.*, 2013). Although in taxa such as snakes, due to a lack of population information and difficulties to detect cryptic biodiversity (because snakes are morphologically more conservative and harder to sample than other reptiles), extinction risk may currently be underestimated (Böhm *et al.*, 2013). This paradigm concerned herpetologists as with the continuation of the present situation, it is possible that part of the snake biodiversity might go extinct even before a proper identification.

In developing countries like Iran, this situation is even more serious. Iran has the most complex and diverse physiography and hence greatest faunal and floral diversity in the southern and arid parts of the Palaearctic realm (Firouz, 2005). During the last half of the 20th century, natural habitats of Iran faced serious environmental damages (for a review see Firouz, 2005). But still, the biodiversity of the country (including snake diversity) has not been sufficiently studied yet. Ficetola *et al.* (2012) suggested that reptile richness in remote areas, such as the Iranian Plateau (including Afghanistan and southwestern Pakistan), as well as parts of Central Asia, may be higher than currently known.

1-1-4- Species concept

At least 22 concepts of what a species is, are in use today, including the familiar biological, ecological, evolutionary, and phylogenetic concepts, among others (for a review see Queiroz, 2007, Mayden, 1997). Many of these are notably incompatible in their accounts of biological diversity (Mayden, 1997). Biologists studying sexually reproducing, living animals, commonly use the biological species concept (BSC), which define a species as a group of interbreeding natural populations that are reproductively isolated from each other (Mayr and Ashlock, 1991). The BSC has received substantial criticism in recent years for issues like the absence of a lineage perspective, exclusion of non-sexually reproducing organisms and important limitations for using this concept in paleontology (Mayden, 1997, Donoghue (1985). The BSC

recognizes the genetic continuity within a species caused by gene flow, but it does not incorporate a view of species existing over evolutionary time.

Currently, at least three different species concepts are identified as including a phylogenetic context (Cladistic Species Concept, Internodal Species Concept, Composite Species Concept). The phylogenetic species concept (PSC) attempts to identify the smallest biological entities that are diagnosable and/or monophyletic (Mayden, 1997). In all versions this PSC is an operational definition, whether one uses diagnosability or monophyly. These concepts also have the ability to recognize species in both biparental and uniparental species. The ability to interbreed is viewed as a shared-primitive attribute and not as having consequences in the recognition of species as taxa (Mayden, 1997). In these concepts, subspecies is not meaningful. There are some problems with the use of these concepts (reviewed by Mayden and Wood (1995)), as for example that because the phylogenetic species concept does not identify species based on the reproductive boundaries between them, it may have the effect of identifying populations connected by gene flow as different species.

Wiley (1978) proposed the evolutionary species concept (ESC) as: “a species is a lineage of ancestral descendant populations which maintains its identity from other such lineages and which has its own evolutionary tendencies and historical fate”. The ESC emphasises that reproductive isolation must be effective enough to permit the maintenance of identity from other contemporary lineages, with morphological distinctiveness not being a requirement. ESC is the only available concept with the capacity to accommodate all known types of biologically equivalent diversity (Wiley and Mayden, 1997). It combines the genealogical basis of the PSC with the genetic basis of the BSC. Because it uses different criteria, much more information may be necessary to define an evolutionary species (Mayden, 1997).

In the ecological species concept (ESC), the species is a lineage (or a closely related set of lineages) which occupies an adaptive zone minimally different from that of any other lineage in its range and which evolves separately from all lineages outside its range' (Van Valen, 1976 in Mayden, 1997). It is an operational definition wherein differences in ecology constitute different, independently evolving species.

1-1-5- Subspecies concept

The subspecies concept has been the subject of decades of controversy (Manier, 2004). Much of the disagreement on subspecies followed from divergent philosophies about its purpose. For many workers after Darwin, subspecies represented incipient species, while for others subspecies represented local populations of a species, showing patterns of local adaptation (Mayr, 1982). In some cases, taxonomic used subspecies for documenting geographic variation in the wrong way and e.g. produced species with as many as 35 trinomials (see Chasen, 1940). Because of Inconsistent applications of the subspecies concept, some scientists suggest abandoning the trinomial altogether (see Wilson and Brown, 1953; McKittrick and Zink, 1988). The majority of attacks on the subspecies concept have resulted from displeasure with its improper application, not from serious flaws in the concept itself. The recognition of diagnosable subspecies allows one to address many questions not easily answered otherwise, ranging from dispersal and migration to local selection and adaptation and biogeographic affinities, yet that goal was lost for many years (Patten and Unitt, 2002). The majority argued, albeit weakly, to keep the subspecies ranking, citing its usefulness in documenting patterns of geographic variation and regions of interest to evolutionary biologists (see Monroe, 1982; Parkes, 1982). Subspecific nomenclature not only provides names for the putative species, but also provides important taxonomic resolution for wildlife management agencies. The subspecies may represent incipient species that may not yet have achieved monophyly, but possess unique morphologies, and are geographically discrete (Mulcahy, 2008). In addition to multivariate morphological statistics (Manier, 2004; Mayr, 1982), phylogeographic studies based on mitochondrial DNA can be used to evaluate subspecific designations in many reptilian species groups (e.g., Wiens *et al.*, 1999; Burbrink *et al.*, 2000) or ecologically associated pattern classes (e.g., Richmond and Reeder, 2002).

1-1-6- Species delimitation and integrative taxonomy

Evolutionary biologists accept the species as separately evolving lineages, but there is disagreements about where along the lineage should be recognized as distinct species (Padial *et al.*, 2010). For species delimitation, there are disagreement about the degree of congruence between different species concepts to identify a population or a group of populations as a separate species (Padial *et al.*, 2010). Integrative taxonomists try to

accommodate new concepts and methods in species delimitations using different species properties.

De Queiroz (2007) proposed that criteria like intrinsic reproductive isolation, diagnosability and monophyly are the secondary species properties and shouldn't be considered relevant to species conceptualization, but only to species delimitation. In this idea, the secondary species properties provide evidence of lineage separation, so the presence of any one of them (if appropriately interpreted) is evidence for the existence of a species (De Queiroz, 2007). Padial *et al.* (2010) refer this approach as integration by cumulation. A major advantage of this approach is that it does not bind species delimitation to the identification of any particular biological property. Taxonomists can thus select and focus on the most appropriate set of taxonomic characters for each group of organisms (Padial *et al.*, 2010). The main limitation of the cumulative approach is that species identification based on or limited species properties can lead to overestimation of species numbers (Padial *et al.*, 2010).

In opposite to cumulation approach, some other taxonomists consider congruence among species properties as a necessary requisite for species delimitation (see Dayrat, 2005). This idea (referred to integration by congruence) states that, concordant patterns of divergence among several taxonomic characters indicate full lineage separation (Padial *et al.*, 2010). This approach promotes taxonomic stability but there is the risk of underestimating species numbers with this approach too (Padial *et al.*, 2010).

1-1-7- Cryptic biodiversity in snakes

Cryptic, or sibling, species are species that are difficult, or sometimes impossible to distinguish morphologically and thus have been incorrectly classified as a single taxon (Beheregaray and Caccone, 2007). Cryptic species³ are found from the poles to the Equator and in all major terrestrial and aquatic taxonomic groups (Pfenninger and Schwenk, 2007). Several lines of evidence underscore the importance of recognizing cryptic species. Conservation planning, bioprospecting, biological control and the treatment of diseases and snakebites all hinge on the identification of species in cryptic complexes. An inability to identify biologically important species therefore hampers our efforts to conserve, study, contain or utilize those (Bickford *et*

³ : The word "cryptic species" in this thesis (as defined in above) shouldn't mix up with another meaning of the word 'cryptic', meaning camouflage or concealment, used to refer special color pattern.

al., 2007). There are several reasons why morphological change might not be correlated with species boundaries or might not be useful in discriminating species, and thus cryptic species may remain undiscovered. There appear to be two general and recurrent themes: cryptic species are either differentiated by nonvisual mating signals (e.g. pheromones) and/or appear to be under a selection that promotes morphological stasis (for a review see Bickford *et al.*, 2007). To resolve this problem, Integrative taxonomists look for concordant changes in more than one characteristic of an organism, and corroboration from independent data (e.g. molecules, morphology or mating signals) is frequently deemed to be good evidence for separating species. The frequency with which cryptic species are uncovered with DNA sequence data (and often subsequently confirmed with morphological and/or ecological data) suggests that molecular data should be incorporated in the research of alpha taxonomists. Different examples of revealing cryptic biodiversity in snakes using molecular data exist, e.g. Marin *et al.* (2013) in Australian burrowing snakes (genus *Ramphotyphlops*), Feldman and Spicer (2002) in dwarf snakes of the genus *Contia*, Rawlings and Donnellan (2003) in green pythons of the genus *Morelia*. Also, there are examples of using both geometric morphometric data and molecular data to uncover cryptic biodiversity in other vertebrates, which shows the effectiveness of using integrative taxonomy in studying cryptic biodiversity, e.g. Hadjisterkotis and Vigne (2006) in the house mouse subgenus *Mus*, and Dobigny *et al.* (2002) in gerbils of the genus *Taterillus*.

1-1-8- Biodiversity of snakes in Iran

Iran can be regarded as a quadri-directional bridge between four major geographical regions: Central Asia, the Indian subcontinent, the Arabian Peninsula and the lands bordering the Black Sea. It is at the crossroads between three distinct floral and faunal regions. In classical terms, Iran is in the Palaearctic biogeographic faunal realm, tangential to the Indo-Malayan realm, and within the influence of the Afrotropic realm (Figure 1-3). Within the Palaearctic, Iran has the most complex and diverse physiography and hence the greatest faunal and floral diversity (Firouz, 2005).

Zoogeographically, species in Iran and adjacent areas of southwestern Asia are distributed between two floral/faunal domains: the Irano-Turanian and the Saharo-Sindian. The species in the area spanning the highlands from Anatolia through Iran and Afghanistan to the

Suleiman range in Pakistan are recognized as Irano-Turanian. Those in the dry southern margins of the Palaearctic are termed Saharo-Sindian (Anderson, 1999).

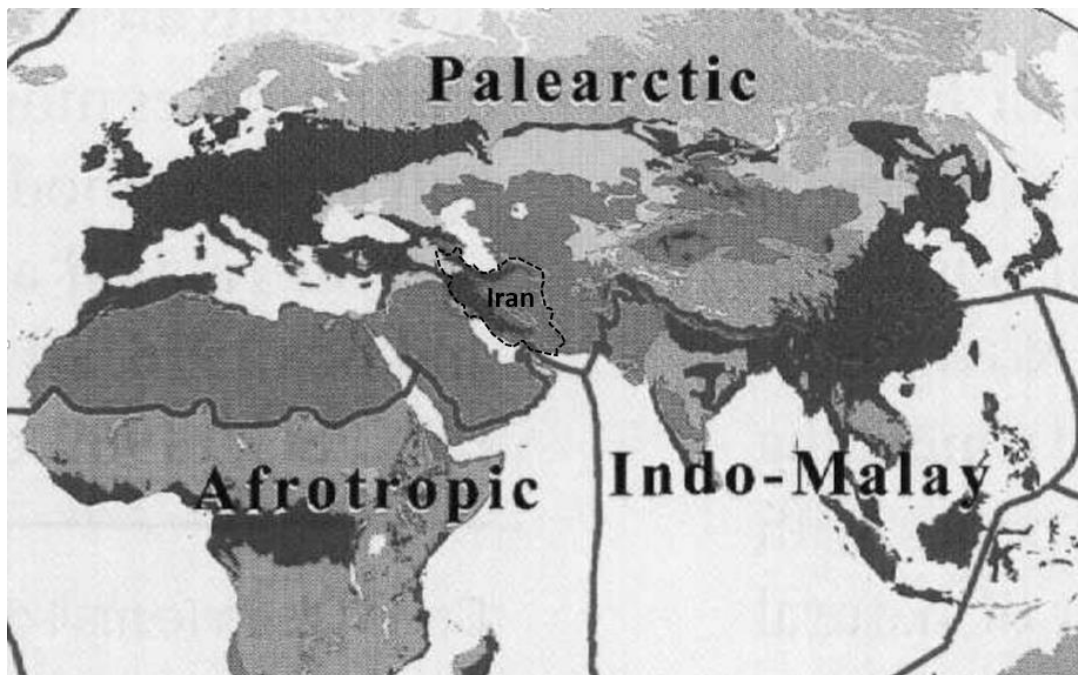


Figure 1-3. The country of Iran with respect to the Palaearctic, Afrotropic and Indo-Malaya biogeographic faunal realms (following Olson *et al.*, 2001).

Diversity of ecosystems in Iran and influence of different faunal realms resulted in a unique diversity of snakes in the country. But unfortunately, there are few comprehensive studies about the biodiversity of Iranian snakes.

There are insufficient data on the biodiversity of snakes in Iran and limited data about cryptic species of snakes in the country. Besides, lack of sufficient scientific field expeditions for studying snakes biodiversity in Iran, another fossorial and secretive behavior of snakes also lead to a restricted knowledge about them, compared to other reptiles and amphibians.

Most of the data on snakes of Iran come from surveys in neighboring countries or adjacent regions that cover the snake biodiversity of Iran too, for example, amphibians and reptiles in the USSR and adjacent countries (Terent'ev and Chernov, 1965), reptiles of Mediterranean countries (Venchi and Sindaco, 2006), amphibian and reptiles of the Middle East (Leviton *et al.*, 1992). Another source of data about snakes of Iran are limited field expeditions or collection examinations reports that cover part of Iran as well (Frynta *et al.*, 1997; Joger, 1984; Haas and Werner, 1969; Schmidt, 1939). There are limited comprehensive studies about specific genera of snakes in the Middle East or western palaearctic that cover Iranian species

too, but most of them suffer from a limited number of specimens originating from Iran (i.e. genus *Montivipera* (Nilson and Andren, 1986); subgenus *Acridophaga* (Nilson and Andren, 2001). The most comprehensive work is Latifi's book, "Snakes of Iran" (1984, 1991 and 2000). This book is a report about the Latifi's snakes' collection in the Razi Vaccine and Serum Institute⁴. He reported 68 species and subspecies of Iranian snakes in his last version of his book. Although he has described the examined specimens of each species very precisely, the taxonomy adopted in the book is old and is needed of a revision. Also, the book suffers from a lack of taxonomic discussion for the problematic species. This problem sometimes leads to taxonomic confusion or misidentification of the noted species (e.g. *Eirenis decemlineatus*, *Eryx jaculus*). Finally, the book is incomplete because it doesn't include snakes species that have been reported from Iran by different herpetologists and only include species that are collected and examined by Latifi himself (e.g. *Coluber* (s.l.) *andreas*, *Lytorhynchus maynardi*).

A literature review of published data on snakes of Iran reveals that there are different problems regarding the taxonomy of snakes in Iran. The most important taxonomic matters deal with species with great morphological variations. Among these snakes, the genera *Hemorrhois*, *Montivipera*, *Macrovipera* and *Platycephalus* can be noted (Schätti *et al.*, 2001; Schätti *et al.*, 1991; Schätti and Agasian, 1985). On the other hand, some snake genera in Iran show very isolated populations with overall morphological similarity. Among these snakes, small-size, species within the genera *Eirenis*, *Lytorhynchus* and *Rhynchocalamus* can be noted (Schmidtler and Eiselt, 1991; Schmidtler and Lanza, 1990; Eiselt, 1971). Another group of snakes which may have unresolved taxonomic matters are species with a huge distribution range. Among these snakes, the genera *Natrix*, *Dolichophis* and *Hemorrhois* are good examples (Gruschwitz *et al.*, 1999).

At this point, the most important questions that come to mind are: exactly how many snake species and subspecies occur in Iran, and what is the taxonomy of them? Also, it is very important to know, whether there is cryptic biodiversity among the snakes of Iran.

⁴ Mahmoud Latifi (who died in 2005) was the Iranian veterinarian who managed snake and scorpion antivenin production in the Razi vaccine and Serum Institute, Karaj, Iran.

Answers to these general questions may give direction to future work on biodiversity of Iranian snakes, as well as the biodiversity of the Iranian plateau. Answers to these questions may give us an estimate about the occurrence of unknown or cryptic biodiversity in snakes or other reptiles of Iran and the Middle East. Certainly, a better understanding of the snake biodiversity in a developing country such as Iran, is essential for future conservation planning and managements.

1-2- Objectives and thesis outlines

1-2-1- General objectives

This study aims to investigate the taxonomy of snakes in Iran and specially assess the occurrence of cryptic species among Iranian snakes. The focus is especially on selected species and genera of Iranian snakes of which there is a great matter of taxonomic confusion. This is the case for species with a broad distribution range, and which occupy very different ecosystems, species that have very different color morphs, and closely related species that are morphologically very similar. We use a combination of taxonomic techniques, from molecular to advanced morphological studies (geometric morphometrics) to clarify inter-population variation and taxonomic status of certain species in the genera *Eirenis* Jan, 1863, *Montivipera* Nilson, Tuniyev, Andrén, Orlov, Joger and Herrmann, 1999 and *Natrix* Laurenti, 1768. Also, we aim to find a logical relation between environmental factors and the taxonomy of these selected snakes species in the country.

The general questions trying to be answered in this thesis, are

- 1- What are the current gaps in the knowledge on Iranian snake taxonomy and what patterns of morphological and biogeographical variation suggest undescribed species that remain to be discovered?
- 2- To what degree can new species be recognized (using phenotypic traits) or is there cryptic diversity among snake species in Iran that may be differ only in their genetic markers?

1-2-2- Outline of the thesis

As the center of focus in this thesis is taxonomic investigations of certain species and genera of snakes in Iran, relevant taxa were detected based on a review (references noted in title 1-1-5) performed on the available data on snakes of Iran. Taxa were then chosen that reflect the taxonomic complexity in the nature of Iran. The arrangement of chapters through the thesis is as follows.

1-2-2-1- In chapters three and four we focus on the taxonomy and inter-population variation in the *Montivipera raddei* group. This eastern species group within the genus *Montivipera* is distributed mainly in northern and western Iran and in adjacent countries. This group is composed of isolated populations of vipers with a relatively high morphological divergence, especially in their dorsal color and pattern. In chapters three and four we focus on the unique color pattern in the *Montivipera raddei* species group. The hypothesis being tested is that the pattern in the *Montivipera raddei* species group is related to environmental factors. In chapter four we review the taxonomy of *Montivipera raddei* species group based on morphological characters. The hypothesis tested here is that different geographically isolated populations of this species group reflect significantly different morphologies with implications on different taxonomic levels.

1-2-2-2- In chapter five we focus on a widely distributed species, *Natrix tessellata*. *Natrix tessellata* is an interesting species with capability for adapting to different semi-aquatic habitats. This species has a global distribution range where in Iran it occupies different aquatic habitats through the northern and western mountains. Distribution, habitat and inter population variation of this species through Iran has not well been studied yet. These data provide a better view on the taxonomy of this widespread species in Iran, as well as the environmental factors which may isolate populations of this species. The hypothesis tested in this chapter is that some of the *Natrix tessellata* populations in Iran may have been isolated geographically with implications on different taxonomic levels.

1-2-2-3- In chapters six, seven and eight the study on the taxonomy of genus the *Eirenis* is presented. The genus *Eirenis* is characterized by morphologically similar species and probable occurrence of cryptic biodiversity. The genus is composed of mainly small size, cryptozoic and

secretive species with isolated populations. The taxonomy of these snakes is very complicated because they are morphologically similar but molecularly very divergent (Nagy *et al.*, 2003). In chapter six we focus on the taxonomy of *Eirenis punctatolineatus*. The species has a relatively broad distribution range from the northern to the southern Zagros Mountains. Our hypothesis in this chapter is that the northern Zagros and southern Zagros populations of *Eirenis punctatolineatus* are taxonomically different. In chapter seven we focus on the taxonomy of *Eirenis medus* species group. This species group has a relatively broad distribution range and we hypothesize that there are populations of the *Eirenis medus* species group that are morphologically different with implications on different taxonomic levels. Finally, in chapter eight we pay attention to the taxonomy of the *Eirenis persicus* species group. This species group has the biggest distribution range in the genus and its taxonomy is very poorly understood. The hypothesis tested in this chapter is that *Eirenis persicus* composes cryptic species.

1-2-2-4- The general discussion includes two major parts. (1) Information from the individual chapters, together with data from the literature is being integrated here to discuss the main processes affecting the biodiversity of the selected species of the genera *Eirenis*, *Natrix* and *Montivipera* in the northern and western mountains of Iran. Occurrence of cryptic biodiversity and the better approaches to identify them are discussed here as well. (2) A synthesis of the taxonomic issues that resulted in the checklist of snakes in the northern and western mountains of Iran.

1-3- Background information about Iran

Iran is located in southwestern Asia. The country has an area of 1,648,000 square kilometres, ranks eighteenth in size among the countries of the world (for a political map of Iran including Iran's provinces, see appendix 5). Iran shares its northern border with Armenia, Azerbaijan, Turkmenistan and the Caspian Sea; the eastern border with Afghanistan and Pakistan; the southern border with the Persian Gulf and the Gulf of Oman and the western border with Iraq and Turkey (Figure 1-4).

1-3-1- Physical geography of the northern and western mountains of Iran

Following Fisher (1968) Iran is generally within the Alpine orogenic zone of Eurasia. Together with its surrounding frame of mountain ranges, this massif separates the Saharan-Arabian portion from the arid zone of the northern hemisphere from its Turanian-Central Asian counterpart. In general, the geographic profile of Iran has been compared with a bowl, with a high outer rim surrounding an irregular and lower interior. The rim is formed by various groups of mountain chains.

The most important mountain range of Iran is the Zagros system. This extends from north-west to south-east, and occupies the entire western part of the country—about half of its total area. The Zagros mountain chain is subdivided in a northern, central and southern portion, of which the central part is the highest part. Parallel to the main Zagros, towards central Iran, there is a chain of younger and lower mountains that are known as the central Iran mountains. At the western side of the Zagros chain, there is a series of low, elevated mountains and hills, named the western Zagros foothills.

Diverging from the northern Zagros in an easterly direction are the northern Iran mountains. The northern Iran mountain, though narrower, are composed of the Alborz mountain chain, Azerbaijan and Kopet Dag mountains. The Alborz mountain chain, which extends in western-eastern direction, is the oldest mountain chain of Iran and is composed of a western, central and eastern portion. The central Alborz portion is the highest one. The Azerbaijan mountains are composed of five, mainly isolated, mountains in northwestern Iran. The Kopet Dag mountains are situated in the northeastern Iran, parallel to the eastern Alborz mountains.

The northern and western mountains of Iran are surrounded by different plains, including the interior basins and small lowlands and coastal plains.

The interior basin or Iran's central plateau is a triangular, highly elevated and arid plain (mean elevation is above 900 meter) that separates the Alborz and Zagros mountain chains. Much of the present surface was once occupied by large lakes. During the Quaternary, water levels have fluctuated considerably. As a result, today the northern and the eastern central plateau is covered by Iran's central Kavir (salt land) and Lut desert. Only the lowest parts of the basins are occupied by residual salt lake, or marshes, and the rest became dry.

In Iran, there are two main lowlands in Iran: the Khuzestan Plain in the southwest and the Caspian Sea coastal plain in the north. The Khuzestan plain to the west of the Zagros mountains is an extension of the Mesopotamia plain. In southern Iran, a thin coastal plain occurs among the coast of the Persian Gulf and Oman Sea.



Figure 1-4. Physiography of Iran. A1: western Alborz, A2: central Alborz, and A3: eastern Alborz mountain chain. Az: Azerbaijan mountains. CK: central Kavir. CI: central Iran plain. CM: central Iran mountains. CS: Caspian Sea coastal plain. EM: eastern Iran mountains. Kz: Khuzestan plain. Lut: lut desert. MK: Makran lowland. Z1: northern Zagros, Z2: central Zagros and Z3: southern Zagros mountain chain (following Fisher, 1968).

1-3-2- Geology of Iran

Africa and the Arabian Peninsula, which had been isolated from Eurasia by the Tethys Sea since the Mesozoic, are thought to have collided with the Anatolian plate approximately 19 Mya (Okay *et al.*, 2010; Harzhauser *et al.*, 2007, Amer and Kumazawa, 2005, Rogl, 1999), although some authors have argued for a much earlier start of the collision (Allen and

Armstrong, 2008). The resulting *Gomphotherium* landbridge facilitated range expansion and divergence of numerous taxa across Afro-Asia (Pook *et al.*, 2009) and allowed faunal exchange between Africa and Eurasia. A drifting movement in the northeast direction of the Arabian Plate resulted in the continuous uplift of the Zagros Mountains, which culminated between 12.4 – 10 Mya (Sborshchikov *et al.*, 1981; Mouthereau, 2011) (Figure 1-5).

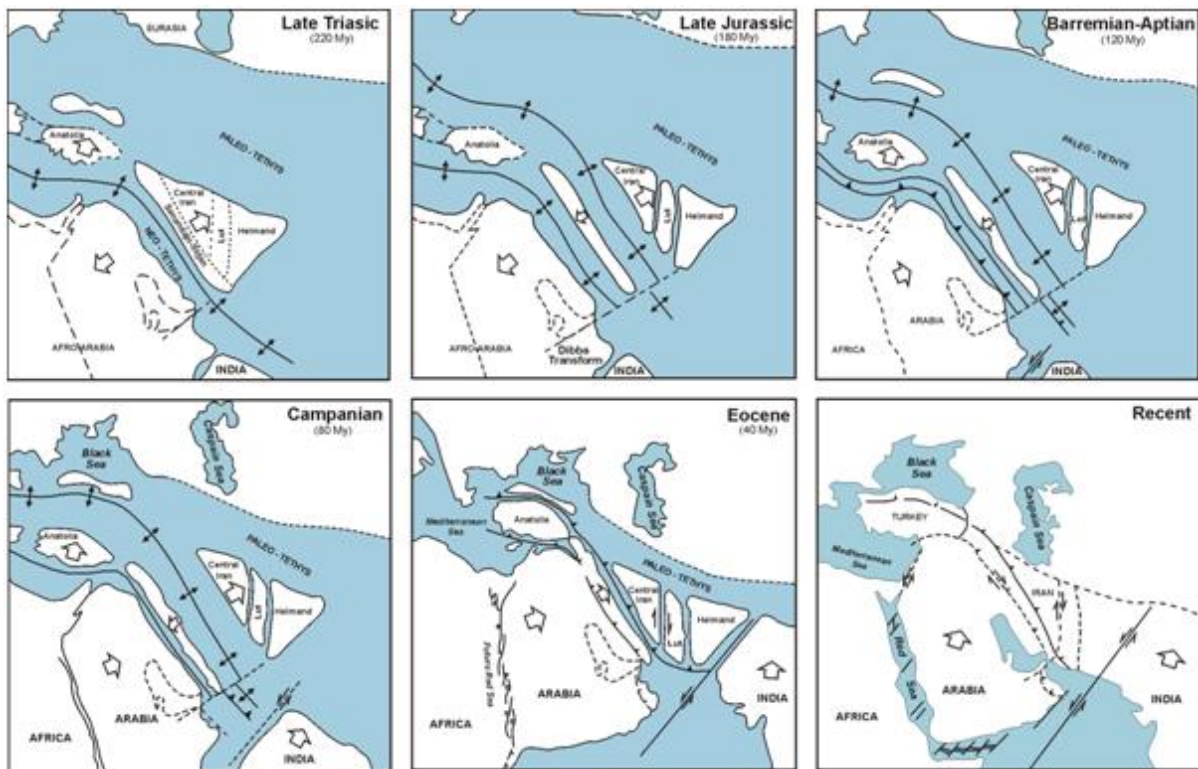


Figure 1-5. Continental Drift of the Arabian Plate beginning in late Triassic to present (modified from Glennie, 1992).

1-3-3- Climate of Iran

The most recent geological era, the Quaternary (see geological time scale <http://quaternary.stratigraphy.org>), is distinguished by the occurrence of glaciations. Most of North Africa and the Middle East experienced an alteration of dry and humid periods, related to the periodic occurrence of glaciations (Schuster *et al.*, 2006). The most recent glaciation, termed the Riss-Würm, reached its maximum about 18,000–21,000 years ago, and in the Sahara and Middle East, this corresponded to a period of higher aridity than at present (Ehlers and Gibbard, 2004). In contrast, after the end of the glaciations, conditions in this region

during the Holocene Climatic Optimum (HGO), 9000 – 5000 years ago, were much more humid than at present (Kaufman *et al.*, 2004).

Nowadays, the Iranian upland is simultaneously subjected to "westerly" weather conditions in the north and to effects of the monsoon in the south (Fisher, 1968). The vast majority of Iran (up to 80%), including the northern, western and eastern highlands and the central plateau are dominated by a Mediterranean climate (Djamali *et al.*, 2011; Zahran, 2010). The northern and western high mountain ranges of Iran greatly facilitate condensation from the air masses associated with the Mediterranean, Caspian, and subtropical oceanic areas; but the resulting precipitation occurs consistently and in quantity only on the outer flanks to the west and north. The northern and western mountains of Iran have dry-summer Mediterranean climates with humid winters (Troll and Paffen, 1964 in Ehlers, 1992). The influence of latitude and elevation is the major factor that controls the distribution of temperature throughout Iran. Also, the topography of mountains is clearly a major factor in the distribution of precipitation in Iran (Fisher, 1968) (Figure 1-6). There are different systems and formulas for producing climate maps. Here we adopted Bio-climatic zones of Iran, available on the FAO website (Figure 1-7). (<http://www.fao.org/ag/agp/agpc/doc/Counprof/Iran/Iran.htm#climate>).

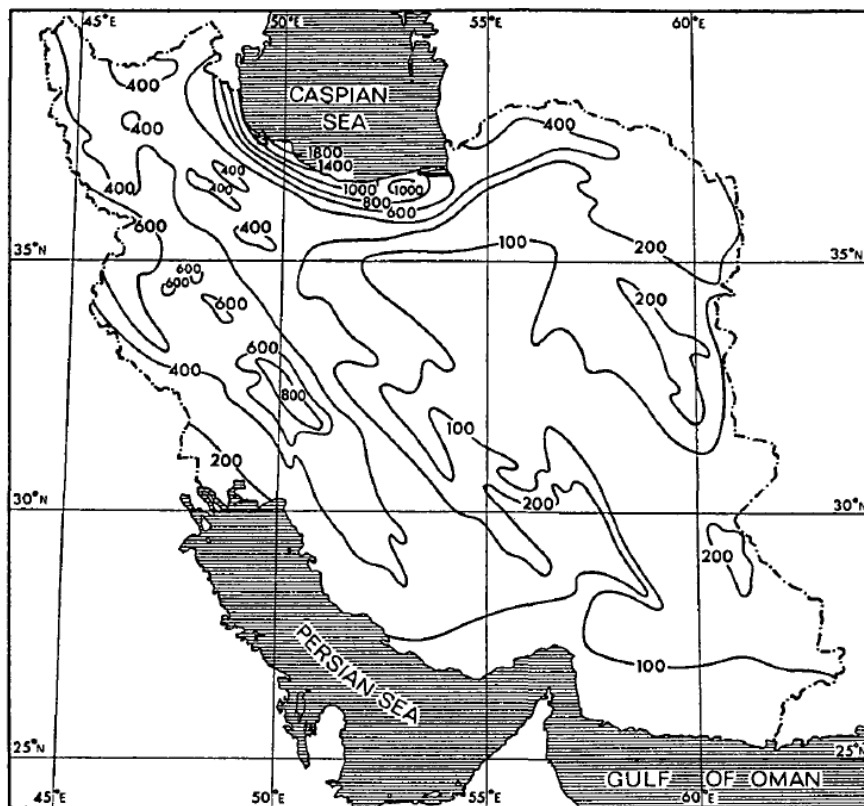
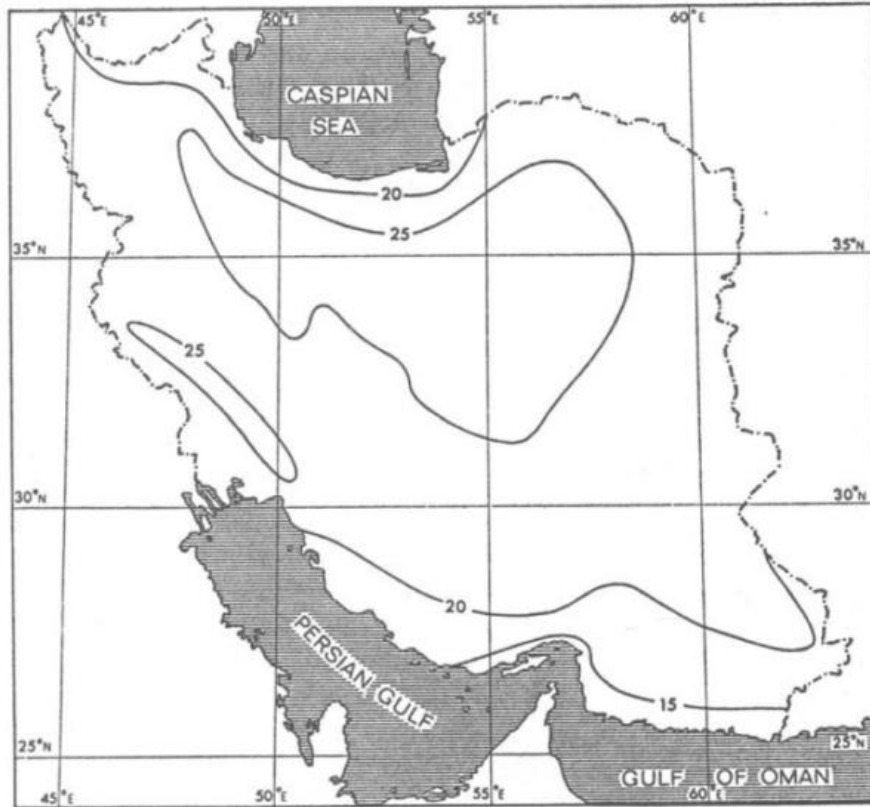


Figure 1-6. Above: Mean annual range of temperature ($^{\circ}$ C) in Iran. Bellow: Mean annual amount of precipitation (millimeters) in Iran.

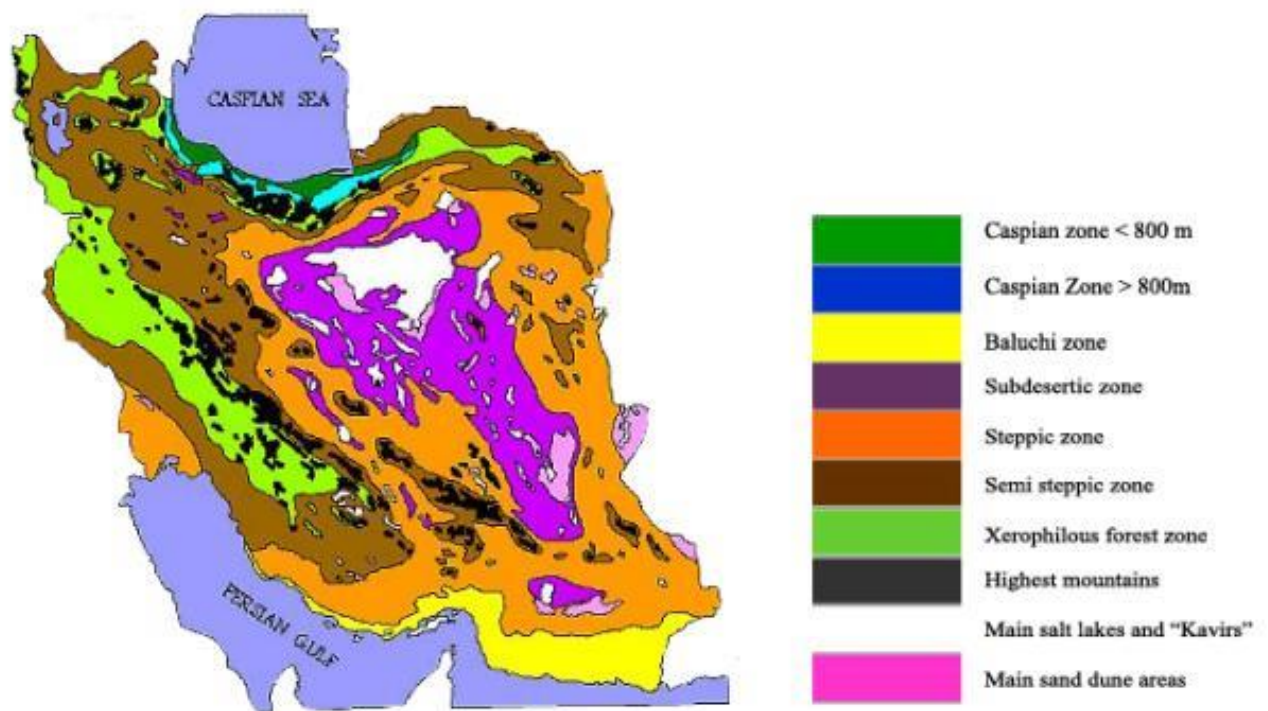


Figure 1-7. Map of bio-climatic zones of Iran.

1-3-4- Vegetation of Iran

Iran has one of the most diversified floras and landscapes in southwestern Asia (Davis *et al.*, 1994; Frey *et al.*, 1999). Three major phytogeographical regions come together in Iran and create a unique biodiversity: the Euro-Siberian region (in the north and northwest); the Irano-Turanian region (central Iran), and the Saharo-Sindian region (Djamali *et al.*, 2011; Zohary, 1973; Akhani, 1998). The flora of Iran contains at least 7300 vascular plant taxa with an endemic percentage of 24% (Rechinger, 1963-2010; Akhani, 2006 in Djamali *et al.*, 2011). This high phytodiversity rate is the result of a complex interaction between different climatic zones and biogeographical regions in a particular orographic context (Zohary, 1973) (Figure 1-8).

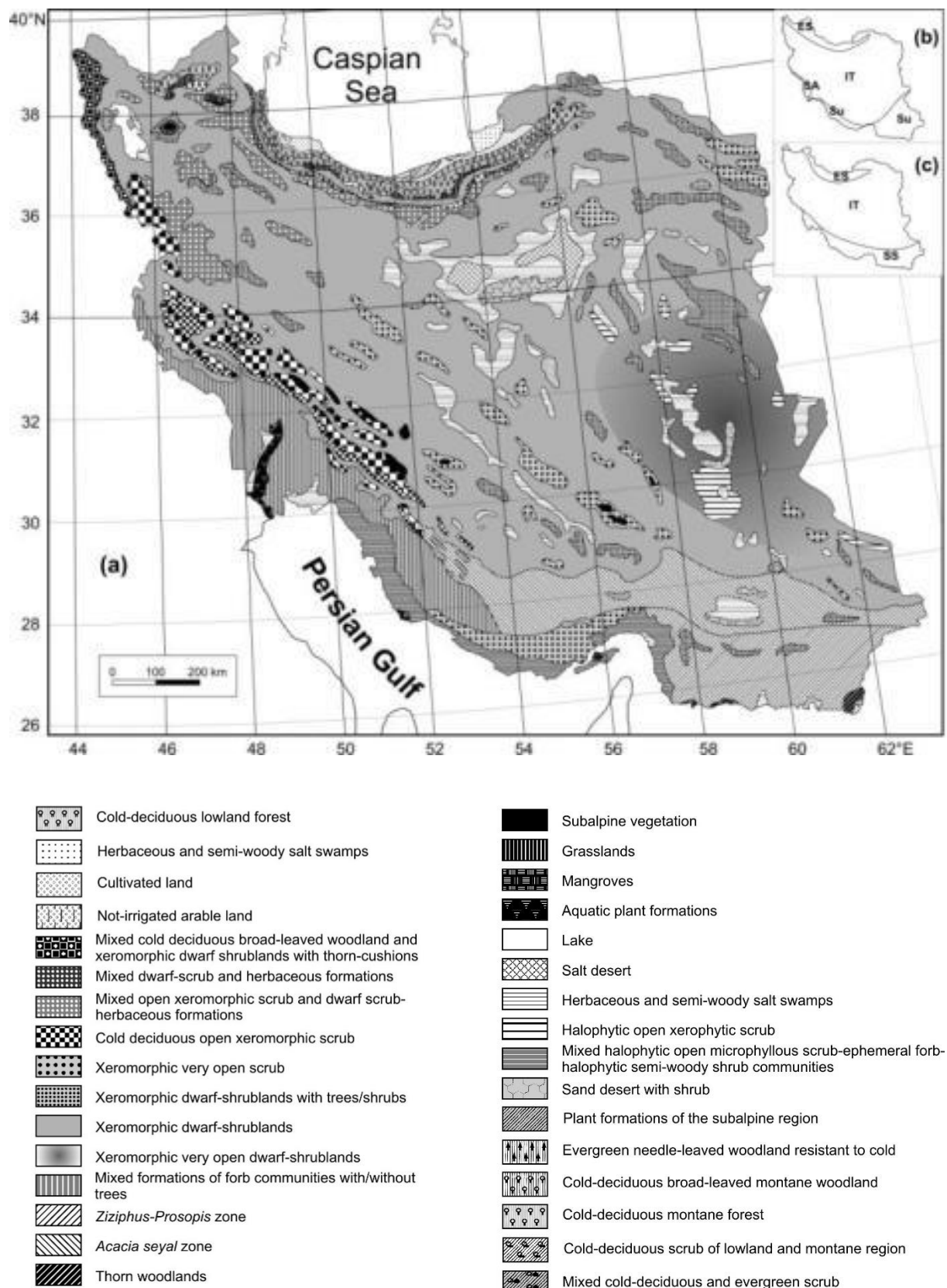


Figure 1-8. Simplified vegetation map of Iran based on Frey and Kürschner (1989).

CHAPTER 2

Materials and Methods

2-1- Study area

The study area in this thesis includes two mountainous ecosystems in Iran, the northern and western mountains. The northern mountains refer to the Azerbaijan, Alborz and Kopet Dag mountains. Western mountain here refer to the main Zagros mountains, the western foothills of Zagros and adjacent mountains of central Iran.



Figure 2-1. The study area including the northern and western mountains of Iran.

2-2- Samplings and collections examination

Through different field expeditions in the northern and western mountains of Iran during the warm season of 2008 till 2011 (Figure 2-2), snakes were collected from different habitats at several times. Specimens were identified in the field and after taking a photo, the sampling locality was documented (coordinates and habitat features) and then snakes were released again. Specimens of the genera *Eirenis*, *Natrix* and *Montivipera* and specimens that couldn't be exactly identified were kept for further studies. Specimens were killed via freezing⁵ and preserved in ethanol 75%. Aggressive killing of any specimen was not done.

These specimens were deposited in the herpetological collection of International Center for Science, High Technology and Environmental Science (ICSTZM) in Kerman, Iran. Also, to examine more snake specimens, collected from the study area, besides visiting local collections in Iran, several travels to important Natural History Museums in European countries were done to study their collections.

List of visited museums out of Iran:

BMNH: British Museum of Natural History, London, United Kingdom.

MHNG: Musée d'Histoire Naturelle Genève, Geneva, Switzerland

MTKD: Museum für Tierkunde in Dresden, Dresden, Germany

ZISP: Zoological Institute, Russian Academy of Sciences, St. Petersburg, Russia

ZMMU: Zoological Museum of Moscow University, Moscow, Russia

ZSM: Zoologische Staatssammlung München, München, Munich, Germany

List of visited universities zoological museums and local collections in Iran:

CBSU: Collection of Biology Department, Shiraz University, Shiraz, Iran

⁵: Specimens collected by M. Rajabizadeh were killed via freezing, now the collector is aware that this is no longer an ethical procedure for killing the specimens. Supposedly some examined specimens, loaned from collections in Iran were killed by freezing or direct injection of ethanol into the heart but the author had no involvement in preserving that specimens. M. Rajabizadeh will use standard Euthanasia methods for killing the specimens (Leary et al., 2013) in future works and recommend it to other colleague in Iran.

DEZC: Department of the Environment of Isfahan Province Zoological Collection, Isfahan, Iran

DHZC: Hormozgan Province Zoological Collection, Hormozgan, Iran

ERP: Eskandar Rastegar-Pouyani personal Collection, Sabzevar University, Sabzevar, Iran

ICSTZM: International Center for Science, High Technology and Environmental Sciences Zoological Museum, Kerman, Iran

MMTT: Muzeye Mellie Tarikhe Tabiei, Tehran, Iran

RI: Razi Institute Collection, Karaj, Iran

RUZM: Razi University Zoological Museum, Kermanshah, Iran

ZMGU: Museum Zoological Museum of Gorgan University, Gorgan, Iran

ZUTC: Zoological Museum, University of Tehran Collection, Tehran, Iran

2-3- Taxonomic studies: Specimens of the genera *Eirenis*, *Montivipera* and *Natrix*, were subjected to detailed taxonomic studies. The following steps were taken to determine the identity of these complex taxa:

- Specimens were primarily examined for morphometric and meristic characters and then inter- and intrapopulation variation was compared with data of type specimens or type locality specimens using descriptive, univariate and multivariate statistical approaches.

- In the cases where morphometric examinations didn't provide enough data for a proper taxonomic decision, geometric morphometric data and molecular data was used to improve taxonomic comparisons. Geometric morphometric analyses were performed to quantify shape variation between populations and reveal even subtle morphological difference between the populations. From that, patterns of variation were expected that could direct towards natural groups. Two mitochondrial and one nuclear gene was sequenced to improve taxonomic comparisons. The sequenced genes were used to generate a phylogenetic tree and check the monophyly of the examine specimens. Also mitochondrial pairwise sequence differences were used to delimit species.

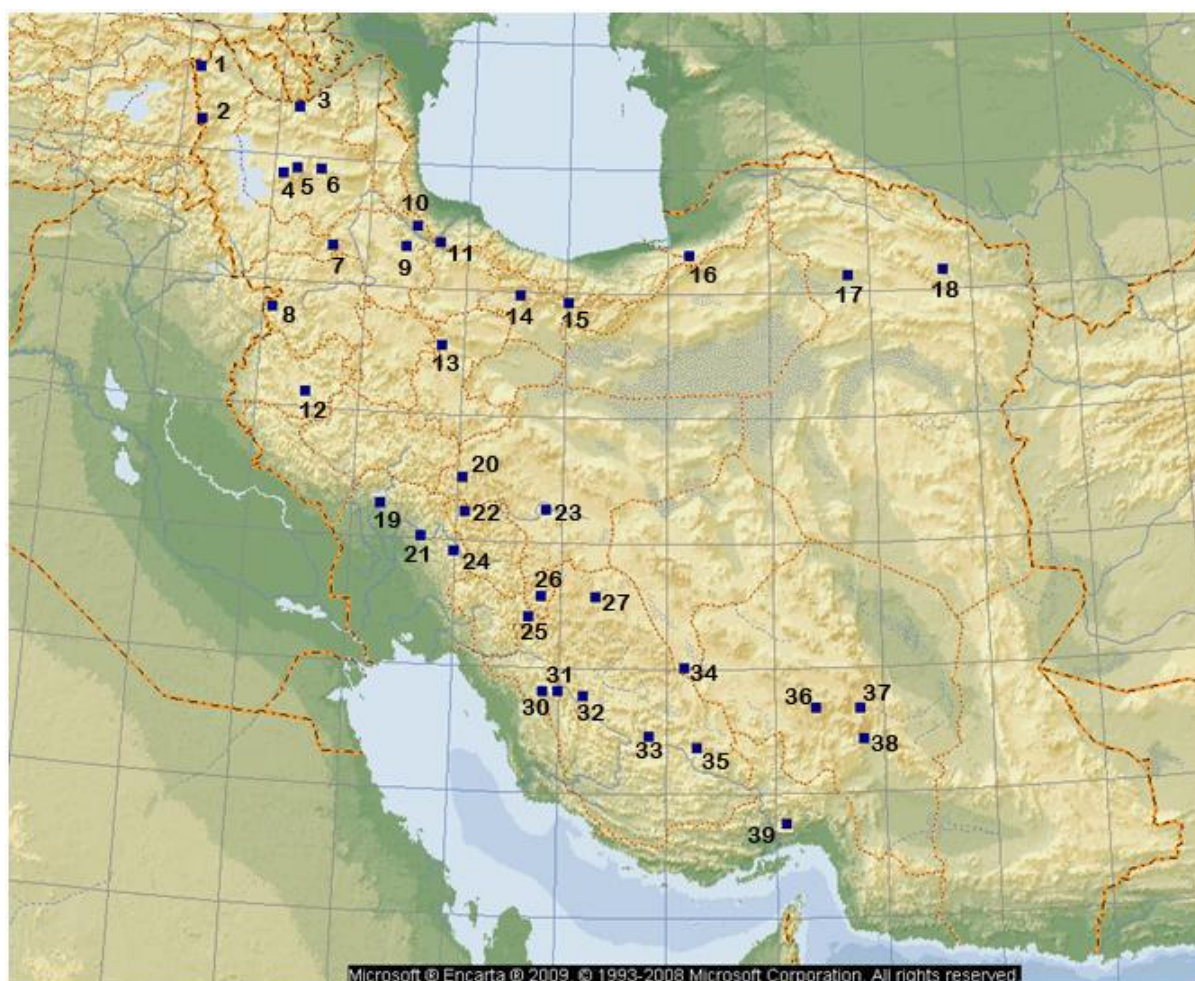


Figure 2-2. Map of visited localities in northern and western mountains of Iran. 1: around Maku city, W. Azarbaijan prov., 2: around Ghotur village, W. Azarbaijan prov., 3: Kiamaki mountains, E. Azarbaijan prov., 4: around Azadshahr city, eastern slope of Sahand mountains, E. Azarbaijan prov. 5: around Lighavan village, E. Azarbaijan prov., around Tikmedash city, E. Azarbaijan prov., 7: around Amirabad village, Tekab mountain, W. Azarbaijan prov. 8: around Marivan city, Kordestan prov. 9: around Khanchay village, Zanjan valley, Zanjan prov., 10: Tarom region, Zanjan prov., 11: around Manjil city, Ghazvin prov., 12: around Kermanshah city, Kermanshah prov., 13: around Gharghabad city, Markazi prov., 14: around Boin-Zahra city, Tehran prov., 15: Lar valley, Tehran prov., 16 around Gorgan city, Golestan prov., 17: around Sabzevar city, Khorasan Razavi prov., 18: around Mashahd city, Khorasan Razavi prov., 19: around Dezful city, Ahvaz prov., 20: Afus region, Esfahan prov., 21: around Vahdati village, Dezful, Ahvaz prov., 22; Kuhrang region, Chahar mahal o bakhtiari prov., 23: around Varzaneh, Esfahan prov. 24: around Izeh, Ahvaz prov., 25: Sisakht region, Yasuj prov., 26; Semirom region, Esfahan prov., 27: around Abadeh city, fars prov., 30: around Kazerun city, Fars prov., 31: around Dashte Arjan village, Fars prov., 31: around Shiraz city, Fars prov., 32: around Seifabad village, Fars prov., 33: around Jahrom, Fars prov., 34: around Khatam city, Yazd prov., 35; Daraab region, Fars prov., 36: Khahr national park, Kerman prov., 37: around Mahan city, Kerman prov., 38: Sarduih region, Kerman prov., 39: Geno mountain, Hormozgan prov.

2-4- Analyses

Laboratory studies were done in different labs both in Iran and in Belgium. Traditional biometry studies were mainly done in the Zoology lab, Department of Biodiversity, International Center for Science, High Technology and Environmental Sciences, Kerman, Iran. Geometric morphometric studies were done in Evolutionary Morphology of Vertebrates research group, Ghent University, Ghent, Belgium. Molecular studies were done in collaboration with Dr. Zoltan T. Nagy in JEMU, Royal Belgian Institute of Natural Sciences, Brussels, Belgium.

2-4-1- Traditional biometry and morphological analysis

Measurements were taken following literature (Dowling, 1951; Thorpe, 1975; Thorpe, 1984, Wüster *et al.*, 1992) (Figure 2-3 and 2-4). A List of the morphological characters and descriptions of them is present in Table 2-1 and 2-2. The measurements were taken using Vernier calipers to the nearest 0.1 mm. For different genera of the examined snakes, morphological examination of at least five specimens was repeated two times to check the accuracy of the examination methods.

The gender of the preserved specimens was determined through a small incision at the base of tail to check occurrence or absence of a hemipenis. If that didn't work, a small incision was made anterior to the anus to check reproductive tract. In museum specimens without gender information, that was not possible to dissect them, a small probe was used to check the anus region (following Laszlo, 1975).

As is common in morphological comparisons of snakes, symmetrical meristic traits of the head were included as the sum of left and right values in the analysis. To standardize morphometric characters with respect to variation in overall size, the ratios of metric characters were used in the descriptive analysis. However, to test for differences in these variables, the raw metric characters were used in an ANCOVA (with total size (TOL) as the covariate). The ratio of head characters to total length was expressed as percentages.

For the analysis of morphological characters, following statistical parameters were used:

1. Descriptive statistical analysis: the morphological examined characters were checked for their normality using a Shapiro-Wilk test), and the central tendencies and the variation around the mean was evaluated using minimum, maximum, mean and standard error (S.E.). For graphic displaying the sample observations of a single variable, boxplots were used. The boxplot used the median and 25% quartiles for ends of the box). The lines (or whiskers) extended to the minimum and maximum values within 1.5 times the spread beyond the quartiles. Large or small values (outliers) greater than 1.5 times the spread outside the closest hinge, were highlighted by star. Descriptive analyses and boxplots were run and generated in SPSS IBM (version 15).
2. Compare mean analysis: to test whether two or more group means differed, the following univariate and multivariate statistical parameters were used. The significance level for all statistical tests was set at $p = 0.05$. For each morphological character, normal distribution (using Shapiro-Wilk normality test) and homogeneity of variance (using Levene test) were tested. Univariate statistics were run in SPSS IBM (version 15) and multivariate statistics were run in PAST (version 2.13) (Hammer *et al.*, 2001).
 - One way analysis of variance (one-way ANOVA): this analysis was used to test significant difference of two or more groups based on individual meristic characters. The LSD post hoc was used to perform a pair-wise comparison among the different groups.
 - Analysis of covariance (ANCOVA): this analysis were used to test significance of metric data between the two or more groups, while statistically controlling for the effects total length that were not of primary interest, known as covariates. LSD post hoc was used to perform a pair-wise comparison among the different groups.
 - Multivariate analysis of variance (MANOVA): This analysis were used to compare multivariate means of several dependent variables between the two or more groups.
3. Principal component analysis: this multivariate analysis was used to convert a set of observations of possibly correlated morphometric and meristic variables into a set of values of linearly uncorrelated variables called principal components. The method was used to summarize the dataset into a smaller number of variables, enabling us to extract the most important source of variation within the dataset and to plot the data for investigating the possible groupings between the specimens. PCA were used to compare between-group versus within-group variation. PCA were done using PAST (version 2.13) (Hammer *et al.*, 2001).

Table 2-1. List of morphological characters examined in snakes of the superfamily Colubroidea

SVL (mm)	Snout vent length from tip of snout to posterior edge of the anal scale
TL (mm)	Tail length from posterior edge of the anal scale to tip of tail
TOL (mm)	Total length or SVL+TL
HL (mm)	Head length from tip of rostral to end of lower jaw
HW (mm)	Head width at its widest part/region
SW (mm)	Snout Width recorded as distance between upper postoculars
SL (mm)	Snout Length from anterior corner of the eye to tip of rostral scale
Ven	Number of ventral scales, following Dowling (1951)
Scd	Number of subcaudal scales on the right side
Sq (a)	Number of dorsal scales at one head-length after head
Sq (m)	Number of dorsal scales at mid body
Sq (h)	Number of dorsal scales at one head-length before cloaca
Pre	Number of preventral scales, following Dowling (1951)
Sup (R/L)	Number of supralabial scales (sum of left and right).
Inf (R/L)	Number of infralabial scales (sum of left and right).
Pre.oc. (R/L)	Number of preocular scales
Po.oc. (R/L)	Number of postocular scales
Sub.oc.a (R/L)	Number of anterior subocular scales
Sub.oc.p (R/L)	Number of posterior subocular scales
Lor (R/L)	Number of loreal scales
Tem1 (R/L)	Number of temporal scales in first row
Tem2 (R/L)	Number of temporal scales in second row
Bet.8.Inf	Number of gular scales between 8 th infralabials in a transverse row
Chin.Pre	Number of gular scales between posterior chin shield and first preventral
L.P.A.Chin	Ratio of length of posterior chin shield to anterior one
P.Chin.Con	Connection or separation of posterior chin shields to each other at the middle
SBP	Number of scales bordering hind parietals
DP	Shape of dorsal pattern, is the dorsal crossbars change to longitudinal parallel lines toward hind body or they disappear at about the tail
VC	Ventral side coloration, is it light or there are dark blotches on the ventral scales?
Lor.s	does loreal scale is deeper than long?

Table 2-2. List of additional morphological characters examined in the family Viperidae.

Incansup	Number of intercanthal + intersupraocular scales, more than 50% above an imaginary line between the end of supraoculars
betsup.e	Number of scales in an imaginary line at end of supraoculars
blspl	Number of scales between last supralabials
InCir	Number of inner circumocular scales
outCir1	Number of outer circumocular scales (round eye and supraocular)
outCir2	Number of outer circumocular scales, round eye (Nilson and Andren, 1986)

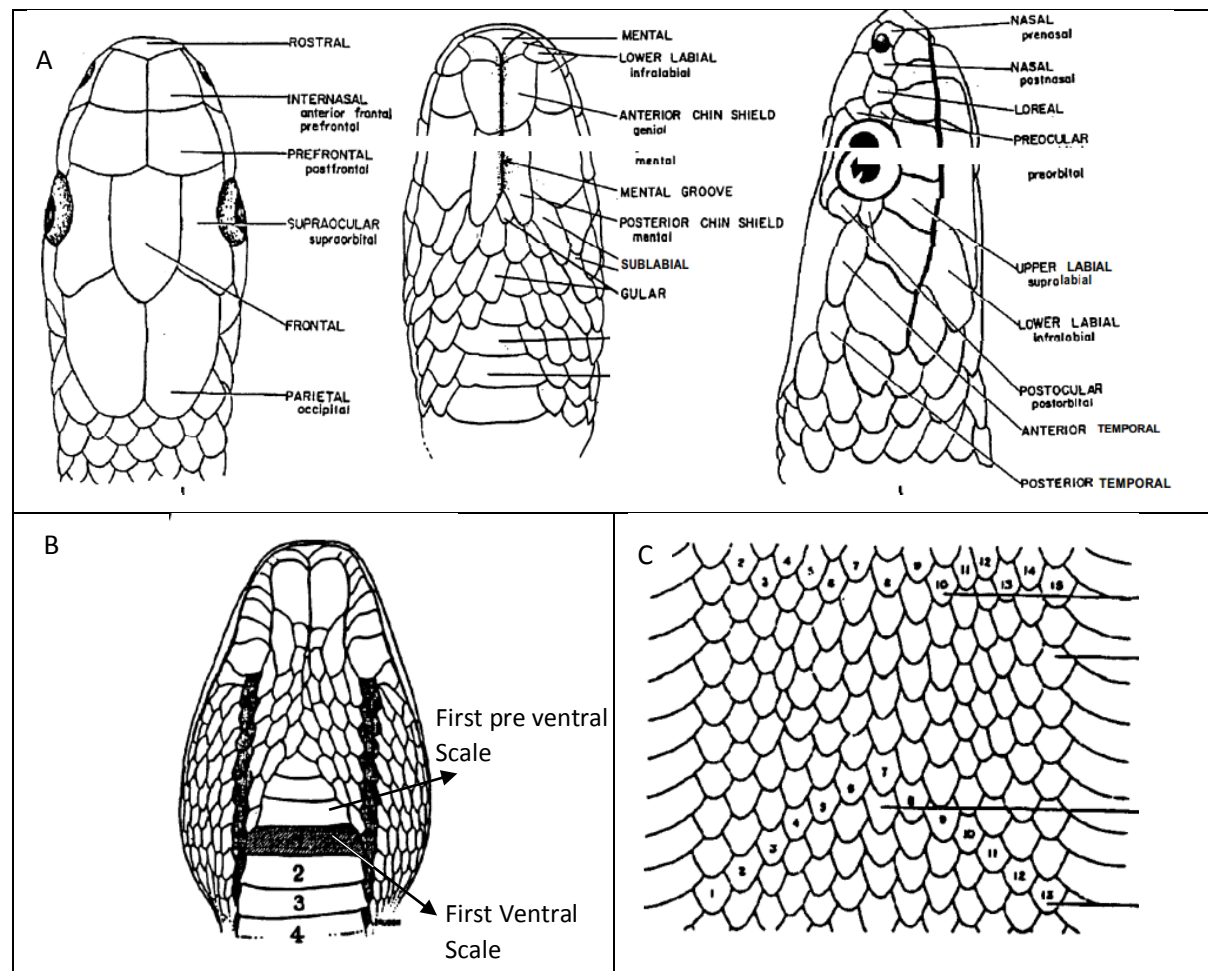
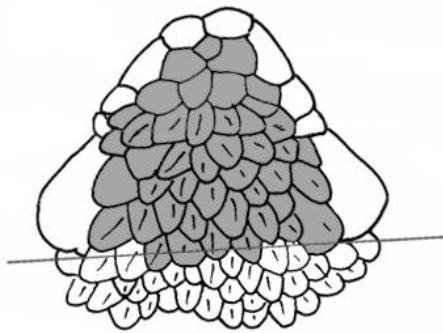
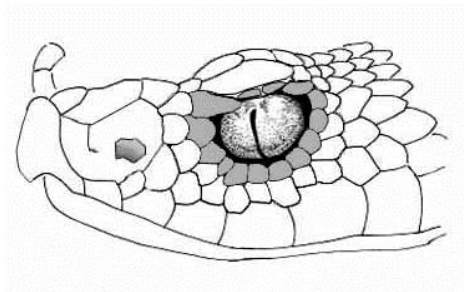


Figure 2-3. Terminology and methodology for naming and counting scales in snakes of the family Colubridae, following Dowling (1951). A: head scales. B: identifying first ventral scale C: methods for counting dorsal scales (source: <http://www.fws.gov/lab/idnotes/IDR951.pdf>).

A



B



C

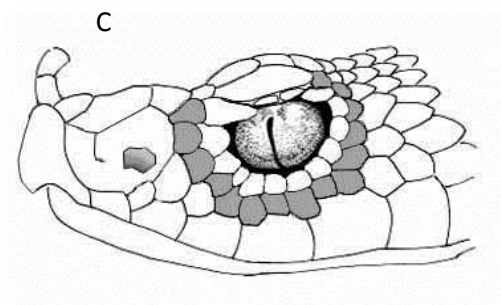


Figure 2-4. Morphological characters in the family Viperidae. A: Number of intercanthal + intersupraocular scales; B: Number of inner circumocular scales; C: Number of outer circumocular scales (outcir1).

2-4-2- Geometric morphometric and geometric analysis

To acquire geometric data from the snake specimens, landmarks were identified on high quality pictures of dorsal views of the head in the specimens. Landmarks were defined as homologous points on the outline of dorsal head scales. Two types of landmarks were used in this study including: type 1 landmark, defined as triple-suture points between the scales; type 3 landmarks, defined as the external points on the outer edge of scales, calculated using a reference line (Zelditch *et al.*, 2012). The landmarks were digitized using tpsDig (version 2) to obtain 2D coordinates (Rohlf, 2010). To avoid observer error, the same picture were digitized two times to assess the associated error with the digitizing. Then landmarks that had obvious errors and couldn't define precisely to remove the observed error, were deleted from the dataset. Also to confirm the accuracy of the digitizing, TpsSmall (version 1.2) (Rohlf, 2003)

was used. Before analyzing shape variation, a generalized Procrustes analysis of the landmarks was performed to remove all non-shape variation (Rohlf and Slice, 1990). In this analysis, first, the centroid of the landmark configurations (representing the shape) are calculated and landmark configurations are scaled to a common, unit size. Then, the configurations are optimally rotated around the centroid to minimize the squared differences between corresponding landmarks (see Rohlf and Slice, 1990). Generalized Procrustes analysis was performed using PAST (version 2.13) (Hammer *et al.*, 2001). To avoid the effect of positioning of the object on the data, multiple pictures of the same specimen were analyzed three times to assess the error associated with the positioning of the specimen and the taking of the pictures. Taking picture of the specimens with obvious error in different analysis were repeated again and if the error didn't remove, the picture were deleted from the dataset. The landmark coordination were analyzed using a between group principal component analysis in PAST (version 2.13) (Hammer *et al.*, 2001) (For more details see title 2-4-1). Shape variation was visualized by deformation grids in tpsRelw (Rohlf, 2003). Multivariate shape variables were obtained from these coordinates, which were subjected to the appropriate multivariate statistical analyses (both tests and ordinations to compare between-group versus within-group variation).

2-4-3- Molecular analysis

Total genomic DNA of the *Eirenis* samples tissues (were taken from the muscle or liver) was extracted using the Mecherey-Nagel NucleoSpin Tissue Kit following manufacturer's instructions. We amplified the same mitochondrial (cytochrome *b* and 16S rRNA) and nuclear loci (*c-mos*) as Nagy *et al.* (2003) (see detailed laboratory protocols therein). Sequencing of the PCR products was conducted using the BigDye Terminator v1.1 Cycle Sequencing Kit on an ABI 3130xl capillary sequencer.

A concatenated data set of cytochrome *b*, 16S rRNA and *c-mos* sequences was analyzed. Alignment was carried out with the MAFFT web server (Katoh *et al.* 2002; Katoh and Toh, 2008). We created seven data partitions: treating all codon positions of both protein-coding genes and 16S separated. In order to find appropriate nucleotide substitution models, we used jModeltest v0.1.1 (Posada 2008) implementing Bayesian Information criterion (BIC). A

Bayesian inference of phylogeny was conducted in MrBayes v3.1.2 (Ronquist and Huelsenbeck 2003), with two runs and four chains each (10 million generations, every 1000th generation sampled, 10% burnin).

Bayesian inference of phylogeny is based on a quantity called the posterior probability of a tree which can be interpreted as the probability that the tree is correct (Huelsenbeck *et al.*, 2001). Posterior probability is calculated by using what are known as Markov chain Monte Carlo (MCMC) simulations to scan through tree and parameter space and calculate the actual probability of the hypothesis (Barton *et al.*, 2007).

2-4-4- Selection of operational taxonomic units

When analyzing patterns of geographic variation, it is essential to discriminate between taxonomically relevant variation due to geographic differences, and taxonomically irrelevant within-locality variation (Wüster *et al.*, 1992). This requires the specimens to be grouped into geographic samples, generally referred to as operational taxonomic units (OTUs). In studies relying on museum material, which often originates from widely scattered localities, it is necessary to pool specimens from more than one locality in order to maximize OTU size. In this study, such OTUs were initially defined on the basis of hypothesized geographic distribution barriers. To avoid pooling specimens of different taxa in one OTU, (1) specimens from different subspecies as defined in literature were split into separate OTUs; (2) specimens of all OTUs were pooled into a single within group principal component analysis based on the correlation matrix, then Individuals of each OTU were checked by visual inspection. If there were specimens, distinctly different than other specimens of their OUT, and had an isolated locality than other specimens of that OTU, these specimens were separated and grouped into a different operational taxonomic unite (OTU).

2-4-5- Criteria used for species delimitation

In this thesis, to distinguish different species, the integration by cumulation approach (following De Queiroz, 2007) was used. To avoid overestimation of species numbers, I tried

to combine all possible species properties (including intrinsic reproductive isolation, diagnosability and monophyly) and thus more lines of evidence for final taxonomic decisions.

Pairwise genetic divergences of the cytochrome *b* gene were used as a supplementary quantitative data for assessment of level of species delimitation in *Eirenis persicus* specimens (chapter 8). Following Johns and Avise (1998); Nagy *et al.*, (2012), average interspecific genetic divergence (p distance) was calculated in *Eirenis persicus* specimens. In addition, average values of genetic distance were calculated among all well-supported species in genera *Eirenis* and *Hierophis*. Species were selected based on well supported clades in the molecular phylogeny of genera *Eirenis* and *Hierophis* (Nagy *et al.*, 2003). These average divergences were then used as specific thresholds for species delimitation in the genus *Eirenis*.

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CHAPTER 3

Analysis of Dorsal Color Pattern Variation in the *Montivipera raddei* Species Complex (Serpentes: Viperidae)

Modified from: Rajabizadeh, M., Yazdanpanah, A. & S. Ursenbacher. (2012). Dorsal pattern variation and sexual dimorphism in *Montivipera latifii* (Mertens, Darevsky and Klemmer, 1967) (Serpentes: Viperidae). *Acta Herpetologica*, 7(1): 13-21.

Abstract:

The dorsal color pattern of 79 specimens belonging to the *Montivipera raddei* species complex, originating from 9 localities across its distribution range, were studied to explore intra and interspecific variation in the color pattern. The analysis revealed that *Montivipera latifii*, *Montivipera raddei* and *Montivipera albicornuta* are significantly different in their color pattern. A correlation analysis showed that there is a significant correlation between the dorsal color pattern of these vipers, and the environmental factors of the habitat, including vegetation structure and stone color. Detailed studies on color polymorphism of *Montivipera latifii* showed that in the Lar National Park, three different dorsal patterns were observed (N=26 specimens) with the following frequency: 50% displayed a complete zigzag dorsal pattern, 15.4% of the individuals exhibited a striped dorsal pattern, and about 34.6% presented an incomplete zigzag dorsal pattern.

Key Words: Geographic variation, color polymorphism, *Montivipera latifii*, *Montivipera raddei* and *Montivipera albicornuta*

3-1- Introduction:

The *Montivipera raddei* species complex is a group of diurnal mountain dwelling vipers, distributed across north, northwestern and western Iran, southern half of Armenia, south of Azerbaijan, Nakhijavan and adjacent places in eastern Turkey. (Nilson and Andrén, 1986). These vipers bear the typical viper's zigzag pattern on their dorsum, in contrast with a dorsal ground color. The *Montivipera raddei* species complex show a remarkable variation in dorsal color pattern, changing from a light dorsal pattern over a dark ground color, to a dark pattern over a light ground color. There also exists variation in the shape of this dorsal pattern.

Although variation in color patterns in populations of the *M. raddei* species complex could reflect an underlying level of biodiversity within this group, so far there has not been a comprehensive study performed on the intra and inter population in variation of color pattern in *Montivipera raddei* species complex. On the other hand, lack of sufficient knowledge about this color pattern variation added even more confusion to the complicated taxonomy of this group of vipers. Schätti *et al.* (1991) generally refused the importance of the color pattern for species delimitation in the genus *Montivipera*, because of the intraspecific variability. But color pattern was used successfully for the taxa delimitation within this genus, as shown by Mulder (1994). In the description of most of the *Montivipera* species, including those of the *M. raddei* species complex, color pattern was used as one of the distinguishing traits by Nilson and Andrén (1984; 1985; 1986).

In this chapter, we tried to explore the intraspecific variation in color pattern across populations of the *Montivipera raddei* species complex. Also, we investigated whether significant differences in color pattern existed between populations in this species complex, and to what degree this could be linked to specific environmental factors. Answering this question could reveal on the one hand whether color pattern in the *M. raddei* species complex is a reliable character for species delimitation, and on the other hand, whether this pattern reflects a local adaption to environmental conditions. A summary of the taxonomy, distribution and habitat of the *M. raddei* species complex, as well as a review on environmental factors that could affect dorsal color pattern in reptiles, is given below.

3-1-1- Distribution and taxa within the *Montivipera raddei* species complex

The *Montivipera raddei* species complex mainly inhabits mountainous valleys and pastures between 1500 to 3000 m elevation throughout the Alborz, Azerbaijan, Caucasus and Zagros Mountains. From a geological point of view, these mountains mainly formed after the collision of the Arabian and Eurasian continental plates in 19 Mya and so different igneous, sedimentary and metamorphic stones with four big volcanos were formed in these ranges (for a review see Rögl, 1999). The climate of the mountain ranges is affected by the Mediterranean macrobioclimate (Zahran, 2010).

Following Nilson and Andrén (1986a) the *Montivipera raddei* species complex consist of four taxa: *M. raddei raddei* (Boettger, 1890), *M. raddei kurdistanica* (Nilson and Andrén, 1986), *M. albicornuta* (Nilson and Andrén, 1985) and *M. latifii* (Mertens, Darevsky and Klemmer, 1967). A phylogenetic study of this species complex, using mitochondrial genes (Stümpel & Joger 2009), rejected the validity of *M. raddei kurdistanica* and casted doubts on the specific status of *M. albicornuta*. In this chapter we follow the Nilson and Andrén (1986) classification and refer to the Zanzan valley population as *Montivipera albicornuta* but in the next chapter, we discuss taxonomy of this species more.

***Montivipera latifii*:** Current knowledge on the distribution of *M. latifii* suggests that this species is restricted to a small area in the central Alborz mountain range (Figure 3-1; Rajabizadeh, 2008; Behruz *et al.*, 2009).

The particularity of this species is a high level of color polymorphism of the dorsal pattern. So far, four different patterns have been identified in *M. latifii*. Most of the specimens present a darker vertebral line connecting with a varying number of alternating or opposing blotches that, together, represent an intensive and well-developed zigzag pattern (Zigzag pattern, Figure 3-2 A). Very occasionally, the dorsal pattern can consist of a one to two scales width, a straight vertebral stripe, without additional blotches (Stripy; Figure 3-2B). Occasionally, the central vertebral line is missing and only the alternating blotches remain, resulting in a diffuse spotty or blotchy pattern (Spotted; Figure 3-2C). Mertens *et al.* (1967) reported a unique specimen with no pattern (Plain). Such kind of patterns seems to be extremely rare in *M. latifii* because, to the best of our knowledge, no other mentions of it have been recorded. In a study carried out by Andrén and Nilson (1979) on the variability of the dorsal pattern in *M. latifii* in

the Lar Valley, no specimen with a completely uniform color was observed, whereas 59% of the individuals presented a well-developed zigzag pattern, 33% had spots or a blotchy pattern and only 8% had a vertebral stripe.

***Montivipera albicornuta*:** This species inhabits the Zanzan valley (Figure 3-1). The dorsal ground color is grayish brown, with a medium brown, black bordered, zigzag band along the back (Nilson and Andrén, 1986) (Figure 3-3).

***Montivipera raddei*:** This species is distributed across easternmost Turkey, extreme northwestern Iran, Naxcivan, southern Armenia and the adjacent area in the Azerbaijan Republic. *M. raddei* has a grayish brown, brownish gray, grayish black or blackish dorsal ground color. The dorsal pattern can sometimes be united into a narrow or broad zigzag band, but consists most frequently of separated, light and often round blotches that sometimes, and especially on the lateral side, form a dark edge (Nilson and Andrén, 1986) (Figure 3-4). *M. raddei kurdistanica* (Nilson and Andrén, 1986) described from around Ghotur village in the Northwestern Iran, based on the difference in color pattern. Nilson and Andrén (1986) argued that *M. raddei kurdistanica* is a very polymorphic subspecies of *M. raddei*, characterized by having all intermediate color pattern morphs, ranging from the typical *raddei* pattern, with light blotches over dark ground color, toward a series of dark blotches on lighter ground color. Molecular phylogenetic studies (Stümpel and Joger, 2009) rejected this subspecies and revealed that specimens referred to *M. raddei kurdistanica*, are different color pattern morphs of *Montivipera raddei*.

3-1-2- Environmental factors

There are examples of correlations between reptile color patterns and substrate color (Michelett *et al.*, 2012; Farallo and Forstner, 2012). Types of vegetation have affected on the color pattern too (Kark *et al.*, 1997; Macedonia, 2001). There are some evidences that in ectotherms, colors and thermal physiology are co-adapted, meaning that dark individuals with a lower skin reflectance are at an advantage under conditions of low temperature thermoregulation (Trullas *et al.*, 2007).

3-2- Materials and Methods

3-2-1- Voucher specimens

For this study, 81 specimens of the *Montivipera raddei* species complex, originating from 9 localities across distribution of this species complex, were studied (Figure 3-1).

To look into the color pattern variation in the *Montivipera raddei* species complex, the dorsal color pattern in 28 *Montivipera raddei*, 27 *Montivipera albicornuta* and 26 *Montivipera latifii* from 9 localities across the distribution range of this species complex were studied (Figure 3-1, 3-2, 3-3 and 3-4) (Table 3-1 and 3-2). Since vipers in the *Montivipera raddei* species complex are difficult to find, it was not possible to assess the intra and inter site color pattern variation, so the available data were grouped for each taxon (following to Nilson and Andrén, 1986) before the analysis.

Data of *Montivipera latifii* were collected between 2006 and 2009 in surveys conducted by M. Rajabizadeh and R. Behruz and pictures taken by amateur Iranian photographers from “Gozal Darre” and Alarm in Lar Valley. For this study, all samples of *Montivipera latifii* were collected in the Lar river valley and side branches of the Lar National Park.

For *M. raddei* and *M. albicornuta*, data collected from living specimens were observed in the nature, freshly killed specimens (i.e. road kills), freshly preserved specimens and high quality pictures. Since preserving materials (i.e. methanol or chloroform) could impact the color of the preserved viper specimens, color data of old preserved specimens were not taken into account in this study. To avoid age effect in the analysis, only adult specimens were examined. Since these vipers are darker during shedding than usual, color data of near to shedding specimens was not considered. Also, because there is no published research about the sexual dimorphism of *Montivipera raddei* species complex in the dorsal color pattern, and we didn't observe a certain intersexual difference too, so the color pattern data of both sexes were used together in the analysis.

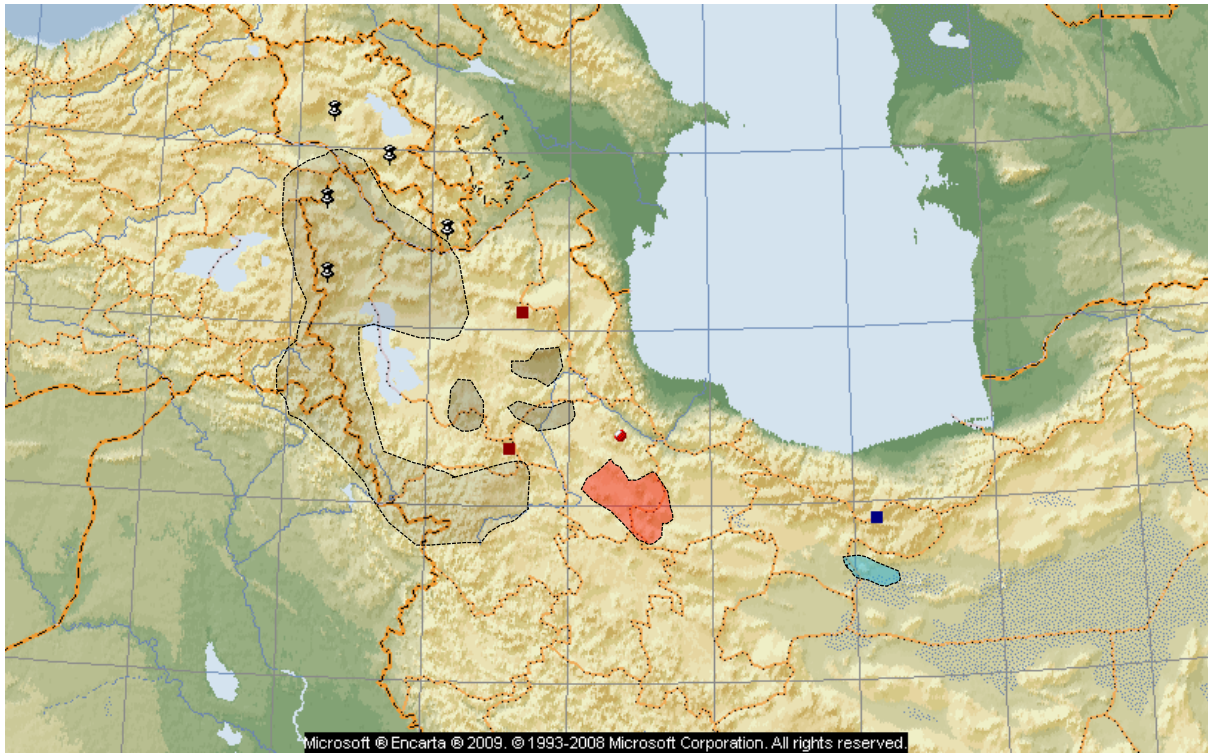


Figure 3-1. Localities of the *Montivipera raddei* species complex (shadow) and localities of the examined specimens (points). Blue: *Montivipera latifii*, in Central Alborz, Red: *Montivipera albicornuta*, in the Zanzan valley. Gray: *Montivipera raddei*, in the north western Iran, Armenia and the north eastern Turkey. Nails show locality of specimens with the *M. raddei* pattern. Squares indicate populations with both the *M. raddei* and *M. albicornuta* color pattern.



Brown / Tan



Brown / Tan



Brown / Tan

Figure 3-2. Different color patterns of *Montivipera latifii* observed in Lar National Park (Iran) (A): stripy; (B): Spotted (incomplete zigzag); and (C): complete zigzag.



Orang / Light Gray



Dark Brown / Cool Gray



Olive / Light Gray



Brown / Light Brown



Blackish / Dark Gray

Figure 3-3. Different colors and patterns of *Montivipera albicornuta*. (A) & (B) are from the same locality in the Tekab mountain. (C), (D) & (E) are frequent color patterns in the Zanzan valley. (F) a black patterned specimen from the Zanzan valley.

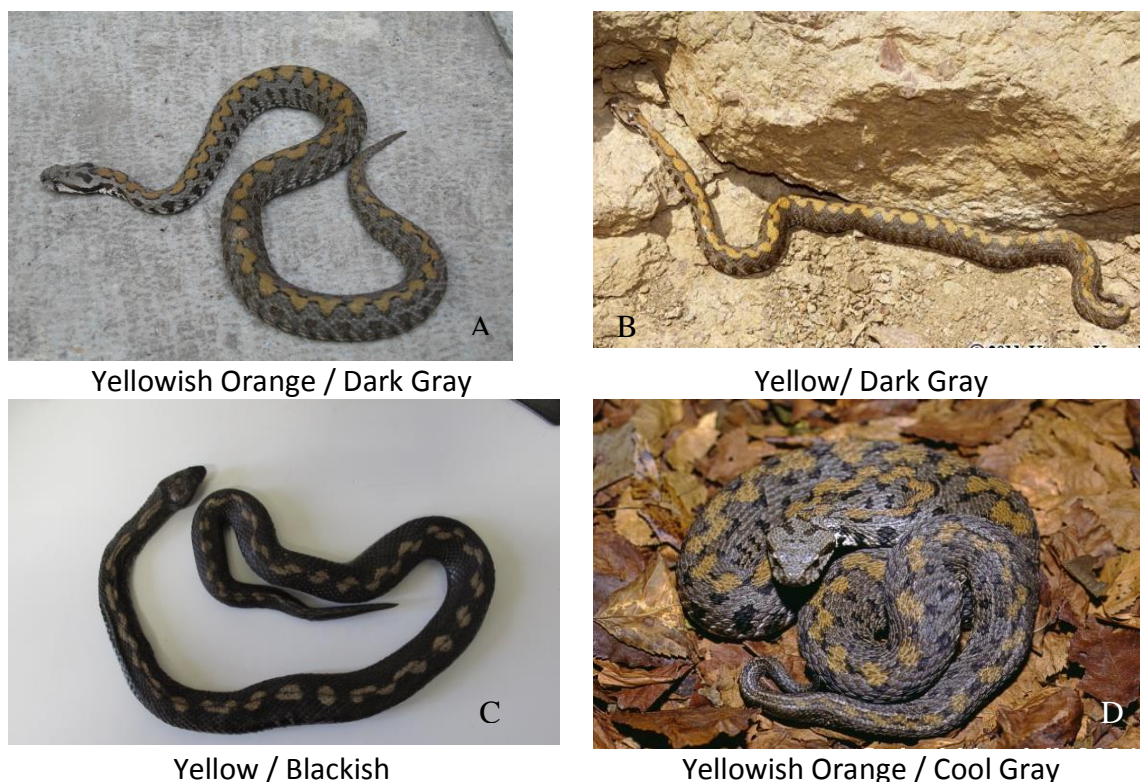


Figure 3-4. Different color patterns of *Montivipera raddei*. (A) from the Maku region, northwestern Iran. (B) from Iran and Turkey bordering mountains extreme north western Iran. (C) from the Shikahokh protected area, Armenia. (D) from Armenia.

For studying the color pattern variation in the *Montivipera raddei* species complex, the dorsal ground color, dorsal pattern color and shape of the dorsal pattern were documented. To do the statistical correlation analysis with environmental conditions, the dorsal ground colors were classified into light, medium and dark categories. To standardize the color names, X11 color names system, presented in (http://en.wikipedia.org/wiki/X11_color_names), was used. This standard color naming system, gives certain name to RGB color values. Based on X11 color names, light dorsal ground color referred to tonalities of tan color. Dark dorsal ground color referred to tonalities of gray, dark gray, cool gray, blackish, as well as grayish brown and grayish olive. Medium dorsal ground color referred to light gray, brown and olive color. Also the shape of the dorsal blotches was classified in two types: roundish and triangular (Table 3-1).

3-2-2- Environmental factors

To investigate the relationship between color pattern variation in the *Montivipera raddei* species complex and the biotic and abiotic environmental factors, two factors with accessible data, of which it has been proven that they are responsible for color pattern variation in reptiles, were including in the analysis: substrate color and vegetation. Climatic factors were not considered in this study, due to the fact that *Montivipera raddei* species inhabit altitudes above 1500 m where a humid, mountainous climate exists. Detailed data of solar irradiation and precipitation of the vipers' locality were not accessible.

Substrate color: In high mountainous habitats, where the *Montivipera raddei* species complex occurs, the substrate is rocky and sandy soil. The habitat is covered by rocky outcrops, gravel fields and sandy soil admixed with differently sized rubble. Color and composition of gravel fields and sandy soils are strongly related to the color of parent rocks. To facilitate the analysis of substrate data, the predominant color of stones in the habitat was regarded as the substrate's color. Petrologic data of the localities were extracted from geology maps of Iran, Turkey and Armenia (www.ngdir.com) and all stone types were listed that occurred in the snake localities (within a radius of 5 km, following Ettling *et al.*, 2013). These stones were categorized to three color types (light, medium and dark), based on the petrologic references and field data. In summary, dark stones were defined as stones similar in color or darker than ophiolite stones. Light stones were defined as stones similar in color or lighter than tuff stones. Medium-colored stones were separated as the rest of stones between the dark and light categories i.e. andesite stones (Figure 3-5).



Figure 3-5. Reference stones used for the classification of stone color. Tuff represents light color stones, Andesite represents medium-colored stones and ophiolite represents dark color stones.

Vegetation: For each viper's locality, vegetation data was documented. As the *Montivipera raddei* species complex inhabits mountainous pastures with a high density of vegetation, only the structure of the vegetation (referring to type of vegetation) was considered in this study, which was grouped in two categories: tree vegetation, referring to forest or shiblik vegetation and bush vegetation, referring to mountain pastures in north and northwestern Iran (characterized by predominant vegetation of cushion shaped bushes, including the genera *Astragalus* and *Acanthophyllum*).

Table 3-1. List of the examined specimens of the *Montivipera raddei* species complex, and their associated color pattern data. Abbreviations: L: light color, M: medium color, D: dark color.

Species	Locality	Nr.	Dorsal Color (pattern color / ground color)	Dorsal Color Abbreviation	Dorsal Blotches
<i>M. raddei</i>	Kotayk prov., Armenia	5 1	orange / dark gray orange / dark gray	L / D L / D	roundish triangle shape
<i>M. raddei</i>	Khosrov reserve, Armenia	4 2	orange / dark gray orange / dark gray	L / D L / D	roundish triangle shape
<i>M. raddei</i>	Shikahogh reserve, Armenia	2	yellow / blackish	L / D	roundish
<i>M. raddei</i>	Maku, Iran	6	yellow / dark gray	L / D	roundish
<i>M. raddei</i>	Ghotur, Iran	1 1 1	yellowish orange / dark gray yellow / light gray dark gray / light gray	L / D L / M D / M	roundish roundish roundish
<i>M. raddei</i>	Tekab mountain, Iran	1 1	dirty brown / cool gray olive / light brown	M / D	roundish
<i>M. raddei</i>	Sabalan mountain, Iran	1 2	olive / light gray brown / light brown	M / M	roundish triangle shape
<i>M. albicornuta</i>	Zanjan valley, Iran	10 11 5 1	brown or olive / light brown brown or olive / light gray brown / grayish brown blackish / gray	M / M M / M M / D D / D	triangle shape triangle shape triangle shape triangle shape
<i>M. latifii</i>	Lar valley, Iran	26	brown / tan or dirty tan	D / L	stripy, spotted and Bare

Table 3-2. List of the examined specimens of the *Montivipera raddei* species complex with the environmental factors of their habitats. Abbreviations: L: light color, M: medium color, D: dark color.

Species	Locality	Topography	Geology	Stones Color Abbreviation	Vegetation
<i>M. raddei</i>	Kotayk prov., Armenia	rocky out croups in hill sides at about 1600 m	Volcanic lava flows and lava floods; basalts andesitebasalts, andesites (100m)	M	dense shibliak vegetation
<i>M. raddei</i>	Khosrov reserve, Armenia	mountain sides at 1700 - 2500 m	Subsuite of the dark tuff-breccias, with the same conditions of middle formation lava flows (400m)	M	Forest
<i>M. raddei</i>	Shikahogh reserve, Armenia	mountain side at about 2100 m	Volcanic lava flows and lava floods; basalts andesitebasalts, andesites (100m)	D	dense alpine meadow above forest
<i>M. raddei</i>	Maku, Iran	river bank at about 1700	Basalt stones	D	shibliak vegetation, predominant Ephedra
<i>M. raddei</i>	Ghotur, Iran	mountain sides at 2000 - 2600 m	Ophiolite stones	D (M,L)	xerophytic bush vegetation with scattered Juniperus
<i>M. raddei</i>	Tekab mountain, Iran	mountain sides at 2300 - 2500 m	Amphibolite stones, adjacent to Gneiss and Andesite stones	D (M,L)	dense alpine meadow
<i>M. raddei</i>	Sabalan mountain, Iran	mountain sides at 2000 - 2200 m	Granite - diorite stones, and andesite lava flows	M	exact locality not clear
<i>M. albicornuta</i>	Zanjan valley, Iran	mountain sides at 2000 - 2300 m	Andesite stones	M	dense xerophytic bush vegetation
<i>M. latifii</i>	Lar valley, Iran	mountain sides at 2500 - 3200 m	Tuff stones	L	dense alpine meadow

3-2-3- Analysis

To evaluate the correlation between color pattern and environmental factors, a Spearman correlation analysis was performed on the categorical data of color pattern and that of environmental factors. Color pattern differences between the examined species were tested using a Kruskal-Wallis test.

3-3- Results

50% of the *M. latifii* from the Lar National Park population has a complete zigzag dorsal pattern, whereas the stripy dorsal pattern was detected in about 15.4% of the individuals and about 34.6% exhibited an incomplete zigzag dorsal pattern. No individual with a “plain” pattern was observed in the Lar Valley.

The Kruskal-Wallis analysis reveals that the dorsal pattern and dorsal ground color, as well as the shape of the dorsal pattern in *M. latifii*, *M. raddei* and *M. albicornuta* is significantly different (Table 3-2). *Montivipera latifii* has a dark color, polymorph (composed of three different morphs) dorsal pattern over a light ground color.

Montivipera albicornuta mostly has a medium color (rarely dark color), with a triangular dorsal pattern over a mostly medium (rarely dark) ground color. *Montivipera raddei* has a light color, mostly roundish (rarely triangular) dorsal pattern, over a dark ground color (Table 3-1).

Table 3-2. *p* value, resulting from the Kruskal-Wallis analysis on color pattern data of the *Montivipera raddei* species complex. The table shows that the dorsal pattern color, dorsal ground color and shape of dorsal pattern are significantly different between the examined specimens of *Montivipera raddei*, *Montivipera albicornuta* and *Montivipera latifii*. The analysis were done pairwise for each variable between the two taxa.

	dorsal pattern color	dorsal ground color	shape of dorsal pattern
df	2	2	2
Sig.	< 0.05	< 0.05	< 0.05

The Spearman correlation analysis showed that the dorsal pattern color, dorsal ground color and shape of the dorsal pattern in all the examined specimens is significantly correlated with stone color and habitat structure of the vegetation (Table 3-3). With respect to color pattern

and habitats of the examined specimen, these results demonstrate that *M. latifii* and *M. albicornuta*, which are characterized by triangular dorsal blotches, always inhabit habitats with bush vegetation, while *M. raddei* with roundish dorsal blotches mainly occurs in habitats with tree vegetation or forests.

With respect to ground color, the data show that *M. latifii* with a light ground color occurs in habitats with light colored stones, that *M. albicornuta* with a mostly medium ground color, inhabits areas with medium colored stones. For *M. raddei* with a dark ground color, it turns out that they inhabit both habitats with medium and dark colored stones.

Table 3-3. *p* value, resulting from the Spearman analysis on color pattern data in *Montivipera raddei* species complex. The Table shows that the variation in the environmental variables (stone color and vegetation structure) is significantly correlated with the variation of dorsal pattern color, dorsal ground color and shape of dorsal pattern in the examined specimens of *Montivipera raddei*, *Montivipera albicornuta* and *Montivipera latifii*.

		Stone color	Vegetation structure
dorsal pattern color	Sig. (2-tailed)	< 0.05	< 0.05
dorsal ground color	Sig. (2-tailed)	< 0.05	< 0.05
shape of dorsal pattern	Sig. (2-tailed)	< 0.05	< 0.05

3-4- Discussion

Results strongly suggest that the observed differences in the color pattern of the *Montivipera raddei* species complex are an adaptive trait. Color patterns in these viper populations seem to be under influence of at least two environmental factors, *i.e.* structure of the vegetation and stone color. Among visual effects, crypsis may represent the major function (and hence selective pressure explaining the adaptive nature) of color pattern variation (Vincent, 1982). Cryptic color patterns may help to avoid detection by predators (Farallo and Forstner, 2012)

and by preys (Götmark, 1987). There are some evidences of disruptive coloration function of the viper's zigzag pattern (Cott 1940; Edmunds 1974; Shine and Madsen 1994), but most of the studies, including recent ones, confirm an aposematic function (Andrén and Nilson 1981; Forsman 1995; Lindell and Forsman 1996; Wüster *et al.*, 2004; Niskanen and Mappes, 2005; Valkonen *et al.*, 2011). It has been shown that even vipers with an aposematic color pattern (e.g. *Vipera berus*) can appear cryptic against their natural background from a distance (Sherrat and Beatty 2003; Tulberg *et al.*, 2005).

There are not enough data about the home range of the different populations in the *Montivipera raddei* species complex. Ettling *et al.* (2013) suggested that the home range for its complete active seasons averages 18.8 ha for males and 32.3 ha for females. Behruz *et al.* (2010) observed in *Montivipera latifii* from the Lar valley that during the onset of spring and autumn, the snakes are mostly observed close to their hibernation sites between rocky outcrops, whereas for the rest of the active season, they were found to be more associated with vegetation.

Dorsal color pattern variation in the *Montivipera raddei* species complex may be the result of a complex trade-off between adaption to vegetation and stones color. In *M. latifii* and *M. albicirnuta*, inhabiting habitats with bushy vegetation, a dorsal ground color is significantly associated with the stone color. But in *M. raddei* that occupying habitats with tree vegetation, all the examined populations, regardless of inhabiting a medium or dark substrate, had a dark dorsal ground color. It seems that tree vegetation, with wide fields of shadow, brings more cryptic advantages for vipers with a dark ground color, even on medium color stones.

Although juvenile specimens were not included in this study, field observations show the coloration in juvenile specimens is paler than adults. Additional field expeditions and researches are needed to analyze the color pattern of juvenile specimens of the *Montivipera raddei* species complex.

In a phylogenetic context (following Stümpel and Joger, 2009), the basal clade, *Montivipera latifii*, is color polymorphic, with three described dorsal patterns within the Lar population. Of those, the dorsal zigzag pattern over a light ground color is the most frequent morph in this species (Figure 3-6).

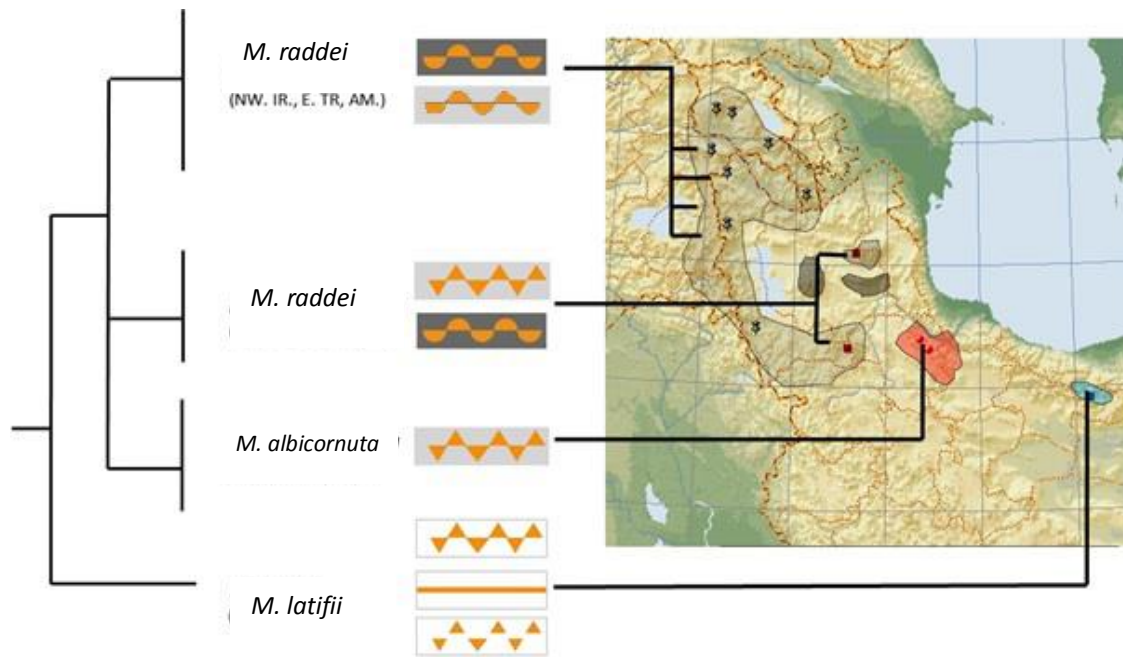


Figure 3-6. Phylogenetic distribution of color pattern morphs in the *Montivipera raddei* species complex. Phylogenetic tree modified from Stümpel *et al.*, (in press)

In the *Montivipera raddei* clade, different populations show different local adaptations in their color and pattern (Figure 3-6). In the easternmost population (Zanjan valley population), which has a distribution and environmental conditions similar to that of the ancestral clade, the dorsal pattern is similar to that of the most frequent dorsal pattern observed in that ancestral condition, but the dorsal color is adapted in relation to the stone color in the Zanjan valley. In the westernmost populations, occupying habitats with a different vegetation structure and different stone color compared to the ancestral condition, both color and pattern have become adapted to the Zanjan valley environment. The *Montivipera raddei* clade, having an isolated distribution across the mountains between the easternmost and westernmost distribution populations (like Sabalan and Tekab), and which shows a mixture of environmental conditions, show both the eastern and western color pattern, thus within a single population (Figure 3-3 A & B).

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CHAPTER 4

Review of Taxonomy and Distribution of the *Montivipera raddei* Species Complex (Serpentes: Colubridae) with Description of a New Species of the Genus *Montivipera* from the Central Zagros Mountains, Iran

Modified from: Rajabizadeh, M., Nilson, G. & H. G. Kami. (2011). A new species of mountain viper (Serpentes: Viperidae) from the Central Zagros Mountains, Iran. Russian Journal of Herpetology, 18(3): 235-240.

Abstract:

Taxonomy of *Montivipera raddei* species complex is not clear fully understood yet. To review taxonomy of this species complex, a total of 67 specimens of the *Montivipera raddei* species complex, including 32 males and 35 females, were examined. Specimens originated from 12 localities in eastern Turkey, southern Armenia, and northern, northwestern and western mountains of Iran. Univariate and multivariate analyses, in both sexes separately, reveal that the central Alborz populations of *Montivipera* are significantly different from populations of Turkey, Armenia, northwestern Iran and isolated mountains of Sahand, Sabalan, Tekab and Kordestan mountains. This study confirms the former classification that refers the central Alborz population as *Montivipera latifii* and that one in northwestern Iran, Turkey and Armenia as *Montivipera raddei*. Morphological analyses show that the Zanzan valley population is significantly different from *Montivipera raddei* populations, but is more similar to *Montivipera latifii*. This result is in contrast with the molecular data that show a close relationship of the Zanzan valley population with *Montivipera raddei*. Since the habitat of the Zanzan valley population is more similar to *Montivipera latifii*, it seems that, the Zanzan valley population is a local population of *Montivipera raddei* species that shows a convergent evolution with *Montivipera latifii*. We suggest to refer to this population as *Montivipera raddei albicornuta*. Morphological characters in a couple of specimens of the *Montivipera raddei* species complex, originating from the Kuhrang region in the central Zagros mountains, represents an unknown species. This species, named *Montivipera kuhrangica* Rajabizadeh, Nilson and Kami, 2011, have the highest number of scales between the last supralabials. Also, the average of total crown scales (intercanthals + interoculars) in this species is higher than in other populations of the *Montivipera raddei* species complex.

Key Words: Taxonomy, *Montivipera raddei* species complex, *Montivipera latifii*, Zanzan valley, Kuhrang region.

4-1- Introduction

The *Montivipera raddei* species complex is a group of mountain dwelling vipers, distributed in north, northwest and western Iran, the southern half of Armenia, south of Azerbaijan, Nakhijavan and adjacent places in eastern Turkey (Nilson and Andrén, 1986). Vipers of the genus *Montivipera*, are characterized morphologically by the presence of a projected, hornlike supraocular scale. The *Montivipera raddei* species complex is distinguished in the genus by the presence of a complete ring of small scales around eye that separate the supraocular scale from the eye, while in the *Montivipera xanthina* species complex, the supraocular is in contact with the eye. (Nilson *et al.*, 1999).

Different ideas have been proposed about the taxonomy of the *Montivipera raddei* species complex. (For a review, see chapter three and Introduction). In previous taxonomic and phylogenetic studies about this species complex, very few specimens of isolated, Iranian populations were studied (Nilson and Andrén, 1985, 1986; Stumpel and Joger, 2009). Part of the taxonomic confusions in this species complex resulted from insufficiently examined specimens. Although there are potential habitats for the *Montivipera raddei* species complex in the Zagros mountains, most of these mountains haven't been explored yet for finding isolated populations of this species complex.

In this chapter, we try to review the taxonomy of *Montivipera raddei* species complex using additional specimens collected from the northern and western mountains of Iran.

4-2- Materials and Methods

Based on extensive field expeditions in the northern and western mountains of Iran, from 2009 till 2010, and collection examinations, a total number of 67 specimens of *Montivipera raddei* including 32 males and 35 females, were examined from 12 localities in the northern and western mountains of Iran, eastern Turkey and southern Armenia (Figure 4-1). Museum specimens originate from ICSTZM, ZUTC, ZMGU, MMTT and MTKD. During field expeditions, we came across an extraordinary specimen of *Montivipera raddei* species complex (following the identification key by Nilson and Andrén, 1986) from the Kuhrang region, located in the high central Zagros mountains (Figure 4-2).

Specimens were examined for 13 morphological characters including three metric characters (SVL, TL, HL) and 10 meristic characters (Ven, Scd, Incansup, blspl, inCir, outCir1, outCir2, Sup, Inf and betsup.e) (see Table 2-2 for definitions). Because patterns of sexual dimorphism in the number of ventral and subcaudal scales had been reported in various populations of *Montivipera raddei* species complex (Nilson and Andrén, 1986; Rajabizadeh *et al.*, 2011), separate analyses were carried out for males and females. For the descriptive analysis and graphic presentation of the metric data, ratio were used: TL/TOL x 100 and HL/TOL x 100 (see Table 2-1 for definitions).

Since the *Montivipera raddei* species complex is distributed across a wide range, and with respect to the scattered localities of the examined specimens in this study, specimens were grouped into five geographic OTUs based on the distance between the populations and specific geographic barriers. Since these vipers are mountain dwelling, wide and arid plains can serve as such a natural barrier preventing distribution across them. These OTUs include, (1) a northwestern OTU, comprising specimens of Armenia and the Iran - Turkey bordering mountains, (2) an SSTK OTU, comprising specimens of the Sahand, Sabalan, Tekab and Kordestan mountains, (3) a zanzan OTU, comprising specimens of the Zanzan valley, (4) a Central Alborz OTU, comprising specimens of the central Alborz mountain, and (5) a central Zagros OTU, comprising specimens of the Kuhrang region in central Zagros (Figure 4-1, 3-2, 3-3, 3-4). The northwestern OTU distribution is separated from that of the SSTK OTU by the Urmia Lake and adjacent arid plains. The Zanzan OTU is isolated from the SSTK one by the arid plains around the Ghezel-Ozan river. The central Alborz and central Zagros OTUs are isolated from each other and from the rest of OTUs by the distance. To reveal character patterns of the different OTUs, descriptive statistical parameters including minimum, maximum, mean and standard error (S.E.) were calculated (Table 4-1). The following analyses were done on the OTUs. To investigate if geographical difference between OTUs is coupled to morphological difference, all OTUs were compared by a between-group principal component analysis (BG-PCA) based on the correlation matrix, using all metric and meristic data. Morphological characters that contribute to a separation of the OTUs in the PCA could then be identified. Principal components were checked up to the level that a cumulative variation of 95% or more is explained. To test whether the OTUs were significantly different, a MANOVA was performed on the OTUs, using characters that were normally distributed and showed a homogeneous variance (tested by Levene's test of homogeneity). The central Zagros OTU was excluded from the statistical analysis because of the low number of samples (2 specimens). For significantly different OTUs, discriminating characters between OTUs were identified using an analysis of variance (ANOVA). Metric characters were used as a ratio in the ANOVA. For the non-parametric characters, analogous non-parametric tests were run to identify those variables that allowed to discriminate between OTUs, *i.e.* an npMANOVA on variables combined, followed by a Kruskal-Wallis on the individual variables.

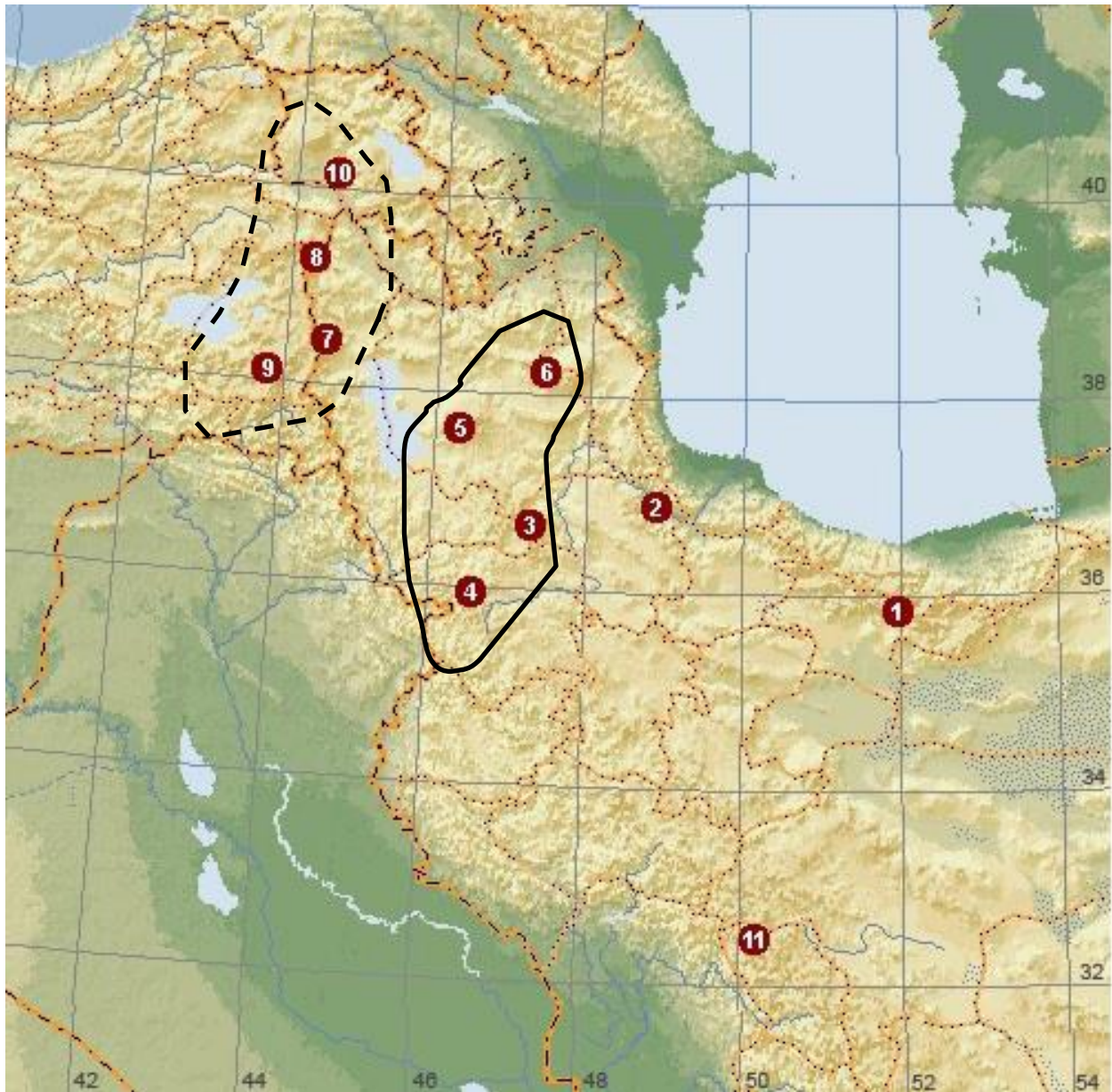


Figure 4-1. Localities of the examined specimens including 1: Central Alborz, 2: Zanjan valley, 3: Tekab mountain, 4: Kordestan mountain, 5: Sahand mountain, 6: Sabalan mountain, 7: Ghotur village, 8: around Maku city, 9: around Yuksekova village, SE Turkey, 10: around Yerevan and sirit cities, Armenia, 11: Kuhrang region in the central Zagros. Northwestern OTU (Dotted line), SSTK OTU (solid line), Zanjan OTU (locality number two), central Alborz OTU (locality number one), central Zagros OTU (locality number 11).

4-3- Results:

The descriptive data of examined characters in each OTU have been presented in Table 4-1. The data of different sexes have been presented independently.

Table 4-1. Descriptive statistic (above: mean \pm S.E., below: range) of the northwestern, zanjan, central Alborz and central Zagros OTUs of the *Montivipera raddei* species complex. The ratios characters were multiplied by 100.

	Males				
	central Alborz	Zanjan	northwestern	central Zagros	SSTK
	9	5	12	2	4
Ven	164.56 \pm 0.88	169 \pm 1.1	173.25 \pm 0.78	164.5 \pm 1.5	168.5 \pm 1.19
	160 - 168	167 - 173	168 - 177	163 - 166	165 - 170
scd	36.56 \pm 0.38	35.2 \pm 0.37	31.17 \pm 0.58	35 \pm 2	31.75 \pm 0.85
	34 - 38	34 - 36	28 - 35	33 - 37	30 - 34
IncanSup	37.11 \pm 1.29	38.2 \pm 1.36	41.5 \pm 1.31	51 \pm 0.7	37.25 \pm 1.11
	30 - 42	34 - 42	32 - 47	44 - 58	34 - 39
betsup.e	9.56 \pm 0.24	9.4 \pm 0.24	9.33 \pm 0.31	10.5 \pm 0.5	9.25 \pm 0.25
	8 - 10	9 - 10	8 - 11	10 - 11	9 - 10
blspl	23.13 \pm 0.2	25.2 \pm 0.2	24.92 \pm 0.4	29 \pm 0.0	25.25 \pm 0.48
	22 - 24	25 - 26	23 - 27	29 - 29	24 - 26
Sup	18.44 \pm 0.18	18.6 \pm 0.4	18.25 \pm 0.18	19 \pm 1	18.5 \pm 0.87
	18 - 19	18 - 20	17 - 19	18 - 20	17 - 21
Inf	22.78 \pm 0.98	24.6 \pm 0.4	24.67 \pm 0.33	25 \pm 1	24.25 \pm 0.48
	15 - 24	24 - 26	23 - 27	24 - 26	23 - 25
InCir	26.44 \pm 0.67	27.8 \pm 0.8	31 \pm 0.63	30 \pm 1	29.75 \pm 1.25
	24 - 30	26 - 30	28 - 36	29 - 31	26 - 31
outCir1	36 \pm 0.85	37.8 \pm 1.02	40.08 \pm 0.42	42.5 \pm 2.5	40.25 \pm 0.85
	32 - 41	35 - 40	38 - 43	40 - 45	38 - 42
outCir2	28 \pm 0.6	29.8 \pm 0.8	30.75 \pm 0.46	32.5 \pm 0.5	31 \pm 0.41
	26 - 32	28 - 32	28 - 34	32 - 33	30 - 32
TL/TOL	9.15 \pm 0.2	8.4 \pm 0.74	7.19 \pm 0.32	8.29 \pm 0.26	8.25 \pm 0.25
	7.97 - 9.95	5.63 - 9.88	4.93 - 8.99	8.03 - 8.54	7.78 - 8.81
HL/TOL	4.47 \pm 0.11	4.63 \pm 0.21	4.66 \pm 0.29	4.44 \pm 0.06	5.4 \pm 0.5
	3.85 - 4.91	4.23 - 5.26	3.21 - 6.39	4.38 - 4.5	4.08 - 6.3

	central Alborz 15	Females		northwestern 10	SSTK 4
		Zanjan 5			
Ven	164.27 ± 0.86 157 - 169	161.8 ± 0.49 160 - 163		171.2 ± 1.42 161 - 178	165 ± 1.47 161 - 168
Scd	32.4 ± 0.74 29 - 39	32 ± 0.84 29 - 34		28.09 ± 0.37 26 - 30	27.5 ± 0.29 27 - 28
Incansup	39.67 ± 1.01 30 - 47	36.6 ± 1.86 32 - 42		42.82 ± 1.19 34 - 48	47.25 ± 1.8 44 - 52
betsup.e	9.6 ± 0.19 8 - 10	9.4 ± 0.4 8 - 10		9.64 ± 0.31 8 - 11	10.75 ± 0.25 10 - 11
blspl	23.28 ± 0.18 22 - 24	25 ± 0.45 24 - 26		25.4 ± 0.54 23 - 28	25 ± 0.41 24 - 28
Sup	18.8 ± 0.3 18 - 22	18.4 ± 0.24 18 - 19		18.45 ± 0.16 18 - 19	18.25 ± 0.25 18 - 19
Inf	23.73 ± 0.25 22 - 26	24.4 ± 0.24 24 - 25		25.5 ± 0.27 24 - 27	25 ± 0.41 24 - 26
InCir	27.27 ± 0.58 24 - 31	27 ± 1.1 24 - 30		30.91 ± 0.99 25 - 37	27.5 ± 1.32 25 - 31
outCir1	37.53 ± 0.45 34 - 40	37.8 ± 0.37 37 - 39		42.18 ± 0.81 36 - 46	40.5 ± 0.65 39 - 42
outCir2	29 ± 0.38 27 - 31	30 ± 0.32 29 - 31		32.27 ± 0.62 28 - 36	31 ± 0.91 29 - 33
TL/TOL	8.67 ± 0.14 7.74 - 9.72	8.61 ± 0.25 8.07 - 9.39		7.12 ± 0.21 5.52 - 8.13	7.76 ± 0.21 7.39 - 8.34
HL/TOL	4.73 ± 0.14 4.09 - 6.32	4.9 ± 0.2 4.41 - 5.53		4.87 ± 0.22 3.7 - 6.27	4.91 ± 0.08 4.7 - 5.09

4-3-1- Multivariate and univariate comparison of OTUs

The scatter plot resulting from the between-groups PCA on the male specimens of the northwestern, SSTK, Zanjan, central Alborz and central Zagros OTUs (Figure 4-2 A,B) reveals that the OTUs diverge from each other mainly by the number of ventral (Ven), subcaudal (Scd), intercanthal + intersupraocular (incansup) and inner circumocular (incir) scales. The Zanjan OTU is located between the northwestern and central Alborz OTUs. The SSTK OTU overlaps with the northwestern OTU. In female specimens (Figure 4-3), similar results are observed, but the Zanjan OTU shows a substantial overlap with the central Alborz OTU. Eigenvalues of the principal components explaining up to 95% of the variation are given in Table 4-2.

Table 4-2. Principal components till level of 95% of variation in male and female specimens of the *Montivipera raddei* species complex.

Male		Female	
PC	% variance	PC	% variance
1	50.53	1	68.37
2	34.1	2	16.61
3	11.98	3	15.01

In male specimens, the following morphological characters were normally distributed and showed homogeneous variance: Ven, Scd, Incansup, Incir, outcir1, outcir2 and TL/TOL. In female specimens, similar characters were normally distributed, with homogenous variance, except for Scd. The MANOVA reveals that males and females from the central Alborz OTU are significantly different from those of the northwestern OTU. Also, males and females from the Zanzan OTU are significantly different from those of the northwestern OTU (Table 4-3). In female specimens, the SSTK OTU is significantly different from the central Alborz OTU too.

Table 4-3. Bonferroni corrected *p* value, resulting from the multivariate analysis of variance (MANOVA) on male and female specimens of the *Montivipera raddei* species complex.

Male					Female				
OTUs	central Alborz	zanjan	SSTK	north west	OTUs	central Alborz	zanjan	SSTK	north west
Central Alborz	-	1.00	0.27	<0.05	Central Alborz	-	1.00	<0.05	<0.05
zanjan	1.00	-	1.00	<0.05	zanjan	1.00	-	0.67	<0.05
SSTK	0.27	1.00	-	1.00	SSTK	<0.05	0.67	-	1.00
north west	<0.05	<0.05	1.00	-	north west	<0.05	<0.05	1.00	-

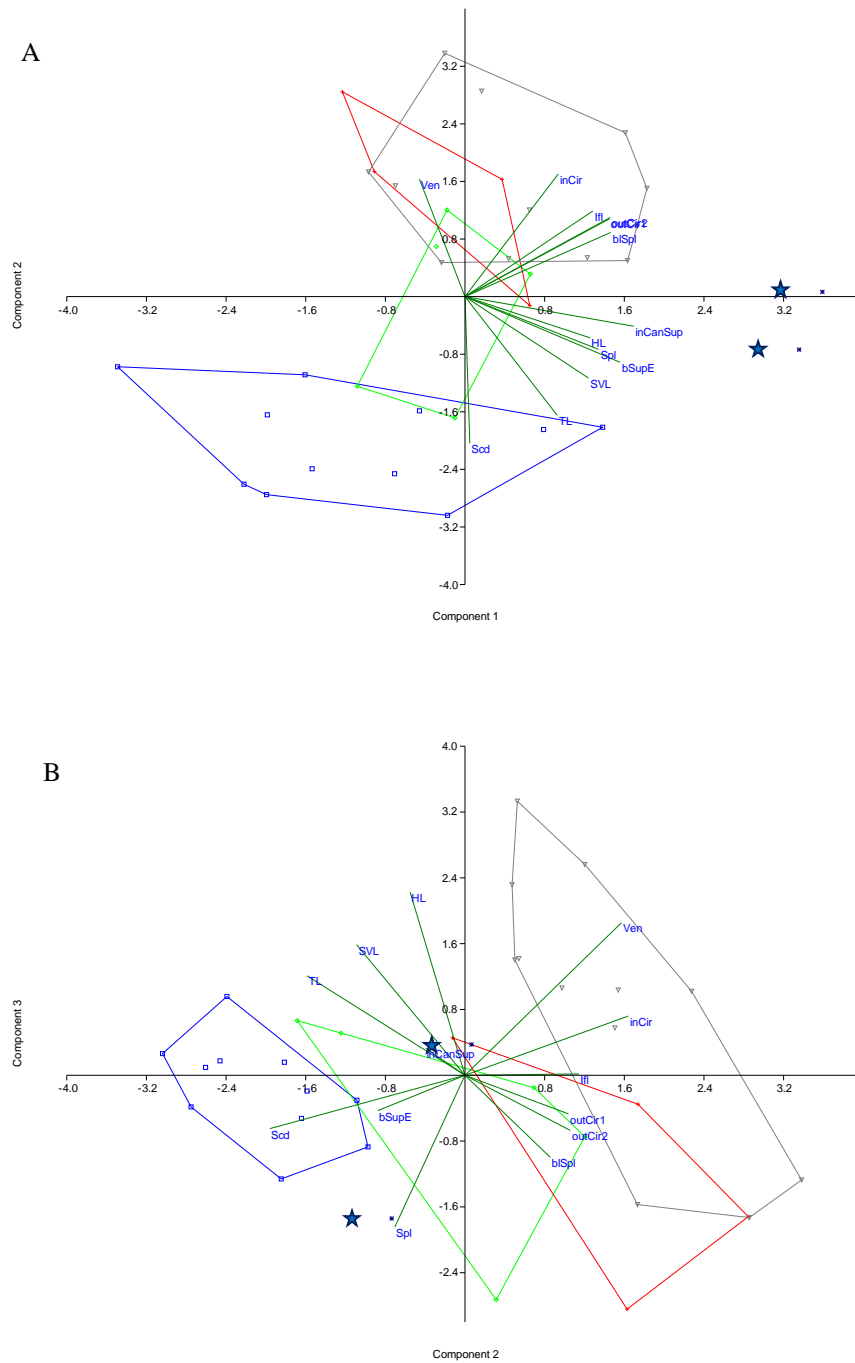


Figure 4-2. Scatter plots resulting from the between-group PCA showing divergence of the central Alborz OTU (blue), the Zanzan OTU (green), SSTK OTU (red), the northwestern OTU (gray) and the central Zagros OTU (blue star) in male specimens of the *Montivipera raddei* species complex. All variables and their contribution (factor loading) in OTU divergence are superimposed on the plots.

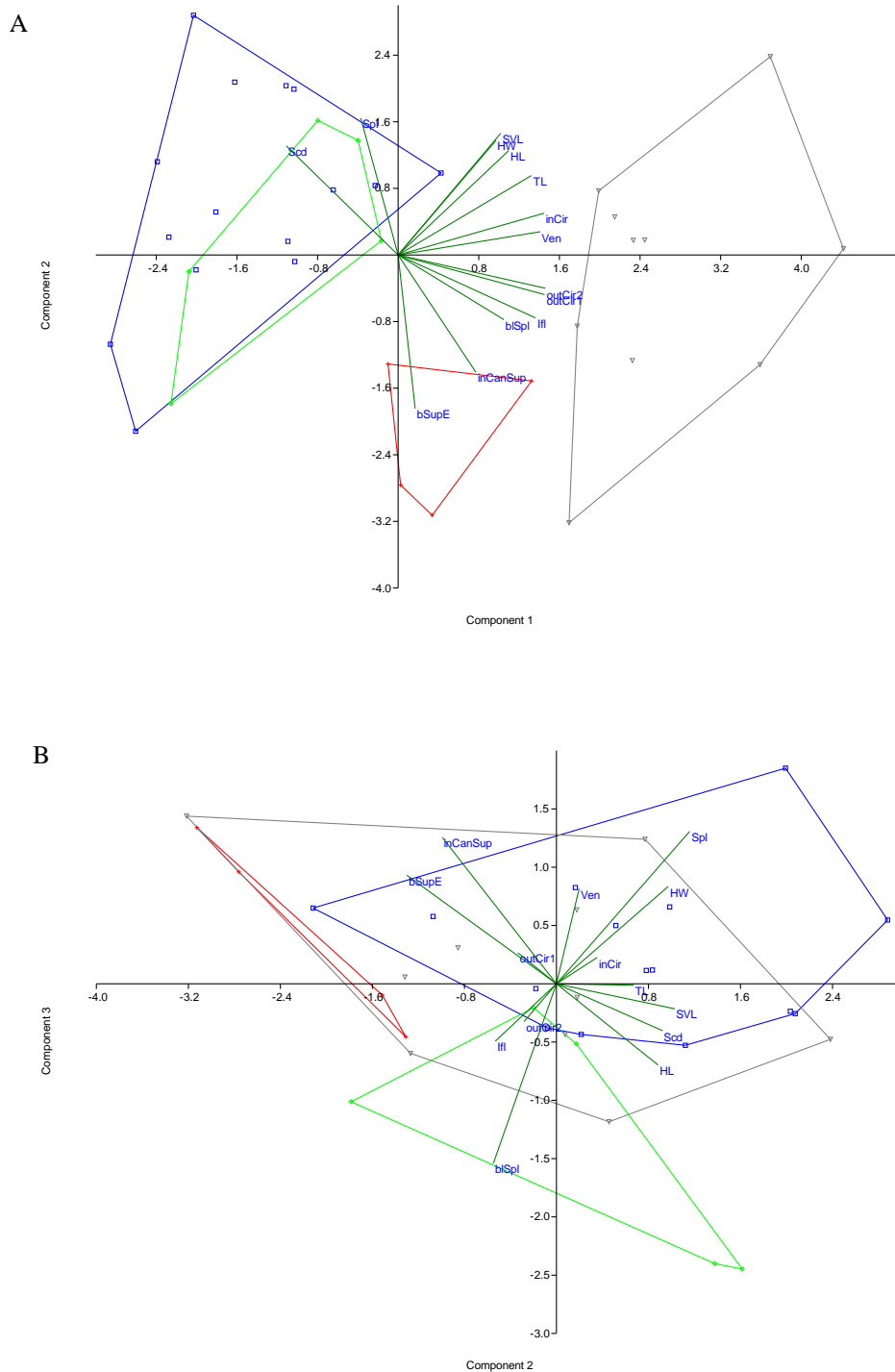


Figure 4-3. Scatter plot resulting from the between-group PCA showing divergence of the central Alborz OTU (blue), the zanzan OTU (green), SSTK OTU (red) and the northwestern OTU (gray) in female specimens of the *Montivipera raddei* species complex.

The npMANOVA showed that none of the OTUs are significantly different. The ANCOVA and ANOVA with LSD post hoc pairwise test between northwestern, Zanjan, SSTK and central Alborz OTUs reveal, in male specimens, central Alborz and zanjan OTUs both have a significant difference with the northwestern OTU in having higher number of subcaudal scales (Scd) a lower number of ventral scales (Ven), a lower number of inner circumocular scales (Incir), a lower number of outer circumocular scale type 1 (outcir1) and a higher tail length (TL). The central Alborz OTU is significantly different from the northwestern OTU in having a lower number of outer circumocular scales type 1 (outcir1) too. The central Alborz OTU is significantly different from the SSTK OTU in having a higher number of subcaudal scales (Scd), a lower number of inner circumocular scales (incir) and a lower number of outer circumocular scales, both type 1 and type 2 (outcir1 and outcir2). The SSTK OTU is significantly different from northwestern OTU in having a lower number of ventral scales (Ven) (Figure 4-4).

In female specimens, the ANCOVA and ANOVA with LSD post hoc pairwise test reveals that the central Alborz and Zanjan OTUs are both significantly different from the northwestern OTU in having a lower number of ventral (Ven), inner circumocular (incir) and outer circumocular scales of type 1 (outcir1), as well as a higher tail length (TL). The Zanjan OTU is significantly different from the northwestern OTU in having a lower number of intercanthals + inter supraoculars (incansup) too. The SSTK OTU is different from the northwestern OTU in having a lower number of ventral (Ven) and inner circumocular scales (Incir). The SSTK OTU differs from the Zanjan OTU in having a higher tail length (TL) too (Figure 4-4). Morphological characters with significant difference between the OTUs are presented in Table 4-4.

Table 4-4. p and F values, resulting from ANCOVA (for metric characters) and ANOVA (for meristic characters) on the characters that were normally distributed and showed homogenous variance, in male and female specimen of the the *Montivipera raddei* species complex. Number of subcaudal scales in female specimens were not normally distributed and were not included in the analysis.

	Males		Females	
	F	Sig.	F	Sig.
TL	9.28	0.00	2.7	0.05
HL	5.55	0.00	39.47	0.00
Ven	19.18	0.00	11.04	0.00
Scd	23.31	0.00	-----	-----
inCanSup	2.67	0.07	6.84	0.00
inCir	8.69	0.00	4.71	0.01
outCir1	8.38	0.00	13.22	0.00
outCir2	5.74	0.00	8.62	0.00

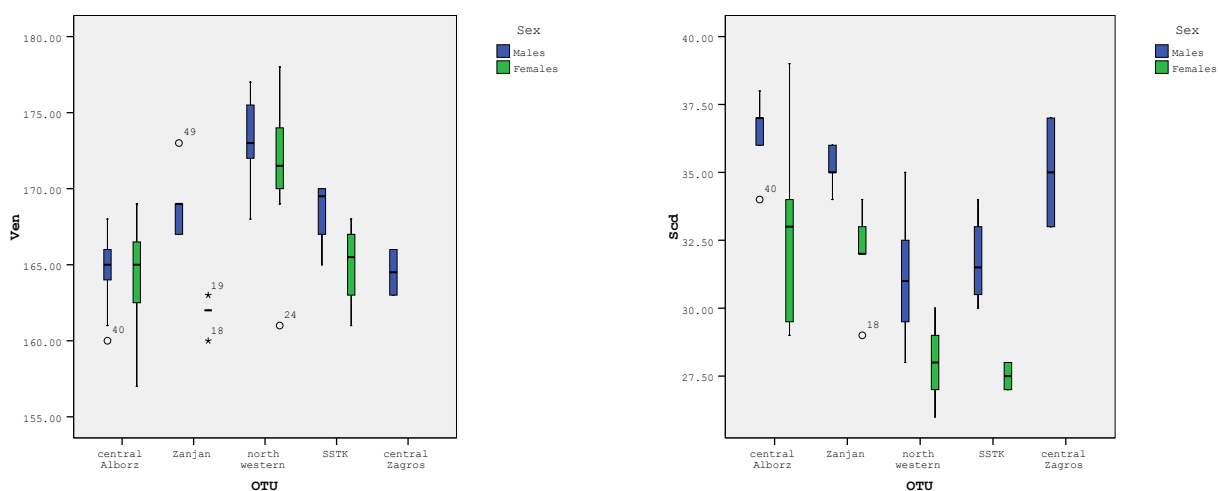


Figure 4-4. Box plots showing the minimum, first quartile, median, third quartile, and maximum of the significant morphological characters across the northwestern, SSTK, Zanzan and central Alborz OTUs. For comparison, the central Zagros OTU was included in the box plots too. The ratio characters were multiplied by 100. Continued on next page.

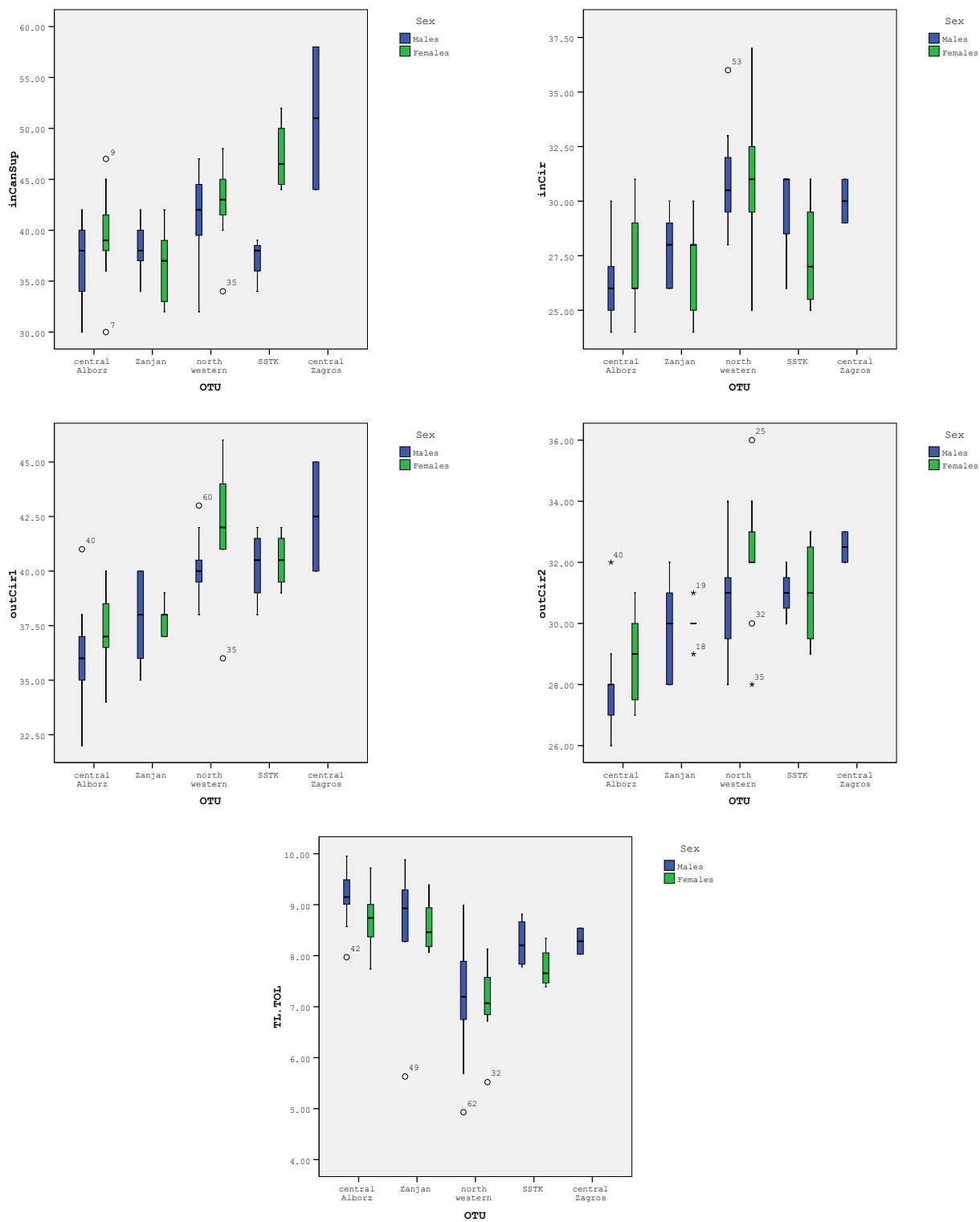


Figure 4-4. Continued. Caption in previous page.

4-4- Discussion

Morphological analysis shows that the *Montivipera* populations in the central Alborz are significantly different from the populations in northwestern Iran, Armenia and eastern Turkey in one metric and four meristic morphological characters for both sexes. These results support the former classifications that distinguish these two groups as different valid species, *Montivipera latifii*, referring to the populations of the central Alborz, and *Montivipera raddei* for the northwestern Iran, Turkey and Armenia populations (Nilson and Andrén, 1986, Schätti *et al.*, 1992). This divergence is also confirmed by molecular data (Figure 4-5) (Stümpel and Joger, 2009, Stümpel, 2012).

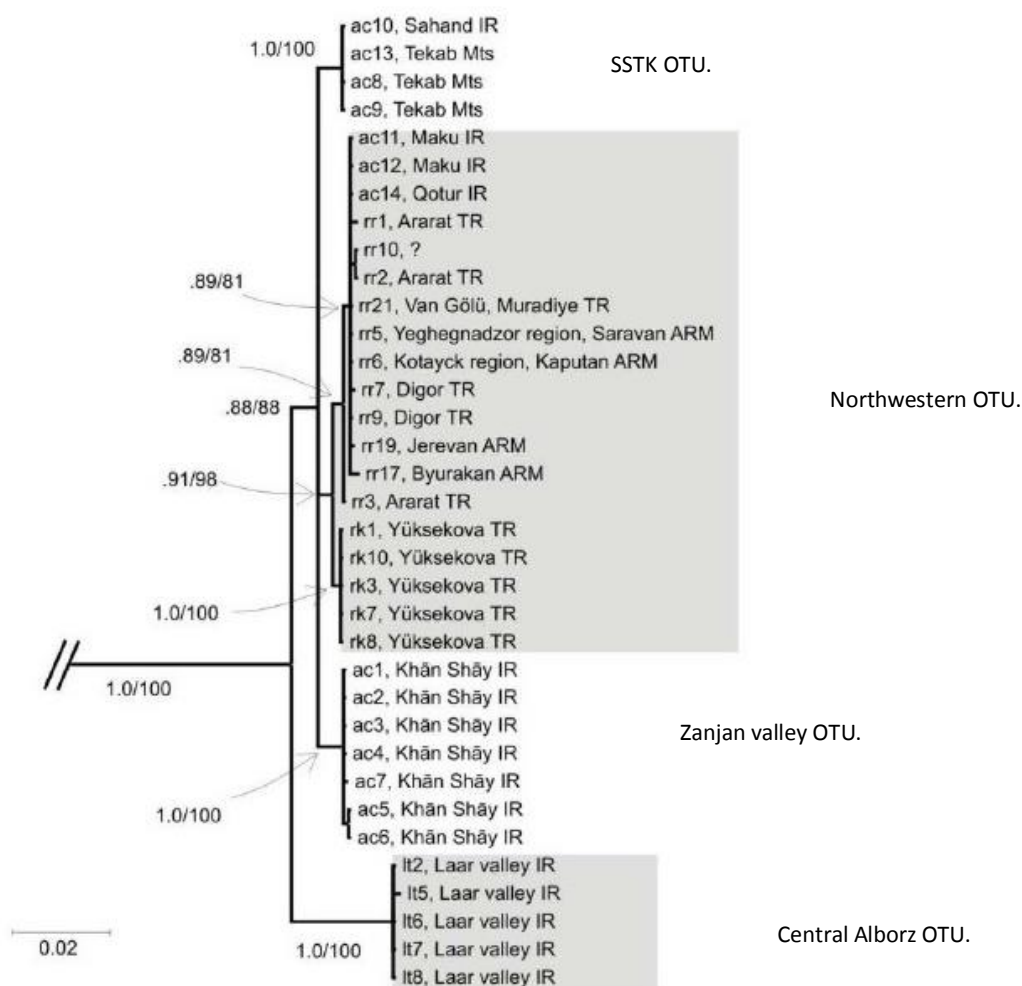


Figure 4-5. Molecular phylogeny of the *Montivipera raddei* species complex based on the three mitochondrial genes, COI, *cytb* and ND5 (Stümpel, 2012). Sister group is the *Montivipera xanthine* species complex.

Morphological data show that the populations of *Montivipera* in isolated mountains of the Sahand, Sabalan, Tekan and Kordestan mountains (referred to SSTK OTU), are more similar to those of the northwestern populations than to those of central Alborz. Female specimens of these isolated mountains (SSTK) differ significantly from the females of the central Alborz population. Geographically these specimens are more close to those of the northwestern populations while ecologically, their habitat is more similar to that of the mountains of northwestern Iran, Armenia and Turkey, than to the highly elevated mountains of central Alborz (see chapter 3). In color pattern traits, the specimens of the Sahand, Sabalan, Tekan and Kordestan mountains have both color patterns that occur in the northwestern and the Zanjan valley populations. Based on morphological (metric, meristic, color pattern) and ecological data, we conclude that specimens of the Sahand, Sabalan, Tekan and Kordestan mountains are isolated populations of *Montivipera raddei*. The observed significantly different character (lower number of ventral scales in both sexes) between these (SSTK) populations and those of the *Montivipera raddei*, may have been the result of the low sample size (only four males and four females were compared), or may reflect the natural geographic variation between these populations. Further data are required to confirm this.

The Zanjan valley population is significantly different from the northwestern Iran, Armenia and eastern Turkey populations (*Montivipera raddei*) in both metric and meristic characters. These populations are more similar to the central Alborz population (*Montivipera latifii*). In color patterns, the Zanjan valley population has a color pattern that is significantly different from both the central Alborz and the northwestern populations. But in a detailed view, the triangular, dorsal pattern of the Zanjan valley population is similar to the most frequent pattern of the central Alborz population, while the dark ground color morph in the Zanjan valley population is similar to that in the northwestern specimens. This indicates that the color pattern of the Zanjan valley vipers shares some features with those of both the central Alborz and the northwestern specimens, but the color pattern components (dorsal pattern color, dorsal ground color and shape of dorsal pattern) of the Zanjan valley viper, occur at significantly different frequencies than the central Alborz and the northwestern populations. From an ecological point of view, the Zanjan valley's habitat is similar to that of the central Alborz in the type of vegetation, but it differs from it in stone color. Also, it is different from the habitats in the northwestern mountains, predominantly in the stone color and vegetation.

The Zanjan valley population is similar to the central Alborz populations in metric and meristic characters, but generally differs from it in color pattern. Although differences exist in the dorsal ground color between the Zanjan valley population and those of the central Alborz, in relation to the environmental factors (see chapter 3), the populations of the central Alborz are color polymorphic with

at least three distinct patterns, while the Zanjan valley population is not. Molecular data show a close relationship of the Zanjan valley vipers with the northwestern specimens (Figure 4-5) (Stümpel, 2012). As with respect to habitats of the *Montivipera* populations in northwestern Iran, Armenia and eastern Turkey, the habitat of the Zanjan valley is more similar to that of the central Alborz (at least, in vegetation structure). Thus we hypothesize that the Zanjan valley population is an isolated population of *Montivipera raddei* that inhabits similar ecological conditions of *Montivipera latifii*. As a result, because the Zanjan valley specimens and *Montivipera latifii* populations in the central Alborz are under similar ecological condition, morphologically there is a convergent evolution between the Zanjan valley and central Alborz vipers, although they do belong to two different phylogenetic lineages. Available molecular data (Stümpel and Joger, 2009, Stümpel, 2012) don't support Nilson and Andrén 's classification (1985) to considering the Zanjan valley population as a valid species. But because there is a significant morphological difference between the Zanjan valley population and the populations of *Montivipera raddei* in northwestern Iran, eastern Turkey and Armenia, we suggest to refer to the Zanjan valley vipers as *Montivipera raddei albicornuta*.

The central Zagros specimens of *Montivipera* are different from the rest of the taxa in the *Montivipera raddei* species complex in having the highest number of scales between the last supralabials (blspl). Also, the average of total crown scales (intercanthals + interoculars) in these specimens is higher than in other populations of the *Montivipera raddei* species complex. Color patterns of these specimens are different from those in other populations of this species complex too. Towards the hind dorsum, the dorsal pattern gradually forms a straight vertebral line (Figure 4-9). Biogeographically, the central Zagros population is highly isolated in the central Zagros mountains and occurs far from the known populations of *Montivipera* in the isolated mountains of Kordestan and Tekab (from SSTK group). Molecular data (Stumpel *et al.*, in press) confirm a divergence of the central Zagros population from the rest of taxa in the *Montivipera raddei* species complex too. Based on all these data, we suggest to consider the central Zagros specimens as a distinct species within the *Montivipera raddei* species complex.

4-4-1 *Montivipera kuhrangica* (Rajabizadeh, *et al.*, 2011)

4-4-1-1- Holotype. Male, ZMGU2203.

4-4-1-2- Terra typica. Tulip valley (Darreye Lale or Dashte Lale) (32°36 N 50°11 E), 8 km north east of Chelgerd village, Kuhrang region, Chaharmahal and Bakhtiari province (the province is located in highest part of the central Zagros mountains), Iran (Figure 4-6). Altitude 2490 m.



Figure 4-6. The type locality in the central Zagros mountains, Iran. The lower parts of the valley are situated at 2400 m altitude.

1-4-1-3- Diagnosis. *Montivipera kuhrangica* (Rajabizadeh *et al.*, 2011) can be distinguished from the other known taxa of the *Montivipera raddei* species complex by a combination of meristic and color pattern characters.

Meristically, it can be stated that the new viper has relatively smaller scales on top of the head and as a result a higher count of head scales compared to other taxa of the *Montivipera raddei* species complex. In addition, it has strongly projected supraoculars. *Montivipera kuhrangica* has the highest number of intercanthal + intersupraocular scales and a higher number of scales between the last supralabials than in any other known taxa of the *Montivipera raddei* species complex (Table 4-1). In addition, the shape of the dorsal zigzag blotches in *Montivipera kuhrangica* is different from that in the other mountain vipers. Towards the hind dorsum, the dorsal pattern gradually forms a straight vertebral line. In the holotype this fusion could be observed on the tail, whereas in additional specimens the fusion of the dorsal blotches can be seen variably in the posterior third of the body.

4-4-1-4- Description of holotype. An adult male viper of the genus *Montivipera* Nilson *et al.*, 1999, with robust body and big and strongly projected supraoculars (Figures. 4-7 and 4-8). In pholidosis: head covered by three apical scales above rostral, three canthal scales between apical (rostral) and supraoculars on both sides with the first two being supranasals. On the dorsal side of the head 18 small scales between canthals (intracanthals), followed by 26 small scales between supraoculars (intrasupraoculars). On the side of the head, eyes are bordered by a complete inner circumocular ring of scales which counted 16 and 13 scales on the right and the left side of head respectively. This circle is bordered by 16 and 18 scales on the right and the left side of head in an outer ring down from the supraoculars. Supraoculars are bordered and surrounded by 10 and 9 scales, loreals are 8 and 8 scales. There is one nasorostral scale, partly fused with the nasal. There are two canthal scales (supranasals) above the nasal; 5 and 5 loreal scales are in contact with the nasal scale. Supralabials are 9 and 11 scales and infralabials are 13 and 13 scales. On the underside of head, the anterior chinshields are more than two times in length to the posterior ones, 5 and 4 infralabial scales are in contact with the anterior chinshield, and there is one divided gular scale (Figure 4-7). Underside of the body is covered by 163 ventral scales which are followed by 37 and 37 (R/L) the pairs of subcaudal scales. The anal scale is divided. There are 23 rows of dorsal scales on the anterior dorsum (one head length after the head), 23 rows of dorsal scales at midbody and 17 rows on the hind body (one head length before anal plate). Among the metric characters, snout vent length (SVL) is 610 mm and tail length is 57 mm. The length of head from tip of the rostral to end of the lower jaw, is 30.0 mm, and width of the head at widest point is 19.6 mm. Regarding the color pattern, the head ground color is light brown and there are two dropletshaped dark brown blotches on the hind part of the head with a pair of small, dark

brown, crescent shaped blotches fused in front of them. A pair of black spots lies in between the later crescent blotches. Scattered dark markings are visible between the supraoculars and on the snout scales. The tip of the strongly projected supraoculars is white. On the side of head, a dark brown strips runs from behind the eye to the corner of the mouth and attaches to a series of lateral, dark body blotches. Supralabials are light except for two minute dark marks below the nasal scales (centered into suture between 2 and 3 supralabials on both sides) and below the eye (centered in sutures between 5 and 6, 3 and 4 infralabials), as well as above the last supralabial, which includes part of postocular dark stripe. In parallel to supralabials black marks, there are black marks on the light infralabials too, located around the sutures between 3 and 4, 6 and 7, and 9 and 10 infralabials. Head scales are marked with scattered fine black spots below. Ventral and subcaudal scales are light but are marked anteriorly with fine black dots. On the ventral scales, on average two to three pale and black blotches on the posterior edge of the scales can be distinguished. The dorsal ground color is light brown with a series of 42 and 44 brown zigzag windings, partly edged by black. Some of the dorsal blotches are triangular or rounded but most are squarish in shape and run in the middle of the dorsum from neck to tail. On the tail the windings fuse together to form a more or less straight vertebral dark strip. Parallel black vertically elongated blotches and brown spots are seen on the lateral and dorsolateral body side in area between dorsal windings (Figure 4-8).



Figure 4-7. Holotype of *Montivipera kuhrangica*: A, dorsal side of body; B, ventral side of body



A



B

Figure 4-8. Holotype of *Montivipera kuhrangica*: A, lateral side of head; B, dorsal side of head

4-4-1-5- Additional specimen. A second specimens was collected by a local snake hunter from around the type locality. Morphological characters of this specimen are as following; adult male, snout vent length, 630.0 mm; tail length, 55.0 mm; head length, 30.0 mm; head width, 18.8 mm; number of ventral scales, 166; number of subcaudal scales, 33; number of intercanthal + inter supraocular scales, 58; number of scales between the last supralabials, 29; number of inner circumocular scales, 15 and 16 on the right and the left side of head respectively; number of couter circumocular scales (type 1), 16 and 17; number of couter circumocular scales (type 2), 23 and 22; number of supralabials, 9 and 9; number of infralabials, 12 and 12. Picture of another specimen, observed in the same locality, was provided by the local snake hunter too (Figure 4-9).



Figure 4-9. Second specimen of *Montivipera kuhrangica* Photo by A. Gholami Arjanaki

4-4-1-6- Habitat. The locality of *Montivipera kuhrangica* is a highly elevated mountain valley, (lower parts of the valley are at about 2400 m and are surrounded by mountains which are up to 3500 m) with a medium to high density of *Astragalus* (named Phrygana Vegetation) and by having a cold mountainous climate (Figure 4-6). Sympatric and predominant species at the type locality are colubrid snakes (*Eirenis* sp. and *Hemorrhois ravergieri*, *Natrix tessellata*, *Dolicophis schmidt*) and lizards of family Agamidae (*Trapelus lessonae*, *Laudakia microlepis*), Lacertidae (*Iranolacerta zagrosica*) and Scincidae (*Trachylepis aurata transcaucasica*).

The habitat of this new viper, *Montivipera kuhrangica* is basically composed of sedimentary stones. This is opposite to the habitat of former known taxa of the *M. raddei* species complex which occur in basically igneous mountains. This difference in origin of mountains has resulted to differences in habitat. In sedimentary mountains like the central Zagros, soil is more abundant and vegetation in the habitat is not as sparse as in igneous mountains.

4-4-1-7- Conservation. Our study shows that the population is very rare and at least during the last 10 years only has been observed at two occasions in this isolated valley in the central Zagros. Despite several visits to the habitat and much searching, only two specimens have been available to us — the type specimen and the one collected by the snake catcher years ago. We know nothing about the population size and with the present conditions of the valley, conservation of this viper must be considered.

There is a very high destructive pressure on the habitat in this valley, especially by many nomads who migrate to the valley during the warm season every year. Nomads use the pastures for grazing sheep to a much higher degree than what is natural for the habitat. In addition, the cultivated areas around the river in the center of the valley block natural altitude migrations of the mountain vipers from hill sides during the cold season to river sides during the warm season.

In the beginning of spring, the valley is a natural habitat of overturned tulips (Genus *Fritillaria*) and every year in the beginning of spring when overturned tulips flower, many visitors come to the valley for outdoor life in all surroundings. This happens accordingly with the end of the hibernation of the vipers, which can be a problem as the vipers and other snakes come out of the shelters in high numbers and are exposed to the risk of mass killing by the many visitors in the valley. In general, snake killings are continued as well by nomads during the whole warm season in the valley.

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CHAPTER 5

Geographic Variation, Distribution and Habitat of the Dice Snake (*Natrix tessellata*) in Iran

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Abstract

The current knowledge of the distribution and habitat of the dice snake, *Natrix tessellata*, in Iran is based on specimens sampled from 69 localities. In Iran, *N. tessellata* is widely distributed across humid lowlands south of the Caspian Sea, the Alborz and the Zagros mountains. Although the species has colonized valleys of large rivers leading through arid plains adjacent to the Alborz and Zagros mountains, its occurrence in arid areas of southeastern Iran is currently indicated but still doubtful. Recent records from the sea shore of the Persian Gulf need confirmation. In order to study the geographic variation of *N. tessellata* in Iran, a total of 66 specimens, including 39 males and 27 females, were examined. The results of univariate and multivariate analyses in both sexes separately show that the males from Fars province (southern Zagros) are significantly different from all other dice snakes in Iran in tail length (TL), head length (HL), head width (HW) and number of ventral scales (Ven). It seems that the morphological variations in these populations are the result of long periods of isolation, mixed with influences by environmental factors of the southern Zagros. But it is known that characters concerning head and body proportions, number of ventral and subcaudal scales vary greatly across regions in *N. tessellata*. So, we feel not confident to draw any taxonomic conclusions before further molecular studies shed light on the phylogeography of this species in Iran.

Key words: *Natrix tessellata*, habitat, morphological variation, Zagros mountains, Fars province, Iran.

5-1- Introduction

Natrix tessellata (Laurenti, 1768) is distributed over a wide range, including central and southern Europe (west to Italy and western Germany, excluding Iberia and France), Crete, Cyprus, Anatolia, Syria, Lebanon, Jordan, Israel and northeastern Africa (Egypt, along the Nile), east to Iraq, Iran, Transcaucasia, central Asia, north to Russia up to 54° north latitude, south to northern and eastern Afghanistan and northernmost Pakistan. Finally in the Northeast across Kazakhstan its distribution ranges into Xinjiang province, northwestern China (Mebert 2011a; Guicking *et al.* 2006; Venchi and Sindaco, 2006; Ananjeva *et al.* 2005; Szczerbak 2003; Gruschwitz *et al.* 1999; Zhao and Adler 1993). One weakly diverged subspecies, *Natrix tessellata heinrothi* (Hecht 1930) from the Ukrainian Black Sea island of Ostrov Zmeinyi (Serpilor Island), has been reported. Its validity is disputed (Gruschwitz *et al.* 1999), but new genetic data show more diversity in this taxon across its wide range (Guicking and Joger 2011; Guicking *et al.* 2009; 2006). This strong genetic differentiation in populations of Iran and other southern populations signals towards the existence of a number of undescribed subspecies.

Because of its semi-aquatic way of life, the habitat of this species is closely connected to water. In its range close to Iran, the former USSR, it was reported from sea shores, riversides and mountains up to 2700 m elevation (Bannikov *et al.* 1977; Terent'ev and Chernov 1949). Although this species is widely distributed in Iran, little information about geographic variation and its habitat in Iran has been provided until now. Exceptions are the books of Latifi (1991, 2000), which provide some information about distribution (based mostly on un-precise locality information) and morphology of this species, and some older literature dealing with the same aspects (Leviton *et al.* 1992; Anderson 1963; Schmidt, 1955). In the current work, we present the geographic variation of morphological characters in *N. tessellata* as well as its distribution and habitat in Iran.

5-2- Materials and Methods

Based on extensive field expeditions and examination of collections, a total of 66 *Natrix tessellata* specimens, including 39 males and 27 females, were collected and examined. The specimens originate from the following zoological museums and collections: ICSTZM, RUZM, ZMGU, CBSU, ERP, ZUTC, MMTT and DEZC.

Specimens were examined for 12 morphological characters including: four metric characters (SVL, TL, HL, HW), two meristic characters (Ven, Scd), and six binary and categorical characters (Sup, Inf, pre, Po, Sub.oc.a, Sub.oc.p) (see Table 2-1 for definitions). Because patterns of sexual dimorphism in the number of ventral and subcaudal scales had been reported in various populations of *N. tessellata* (Mebert, 2011b; Gruschwitz *et al.* 1999; Mebert, 1993), separate analyses were carried out for males and females. For the descriptive analysis and graphic presentation of the metric data, ratios were used: TL/TOL x 100, HL/TOL x 100, HW/TOL x 100 (see Table 2-1 for definitions).

Since *N. tessellata* is distributed across a very wide area in Iran, and because the examined specimen are geographically scattered, the specimens were grouped into OTUs geographically. To reveal character patterns of the different OTUs, descriptive statistical parameters including minimum, maximum, mean and standard error (S.E.) were calculated. The following analyses were done on the OTUs

To investigate if geographical difference between OTUs is coupled to morphological difference, Using characters that were normally distributed and showed a homogenous variance (including SVL, TL, Ven, Scd), all OTUs were compared by a between-group principal component analysis (BG-PCA) based on the correlation matrix. Morphological characters that contribute to a separation of the OTUs in the PCA could then be identified. Principal components were checked until the level of 95% of total variation. To test whether the OTUs were significantly different, a MANOVA was performed. For significantly different OTUs, discriminating characters between OTUs were identified using an analysis of covariance (ANCOVA) for metric characters (total length as covariate) and analysis of variance (ANOVA) for meristic characters. For the non-parametric characters analogous parametric tests were run to identify those variables that allowed to discriminate between OTUs (NPMANOVA on combined variables followed by a Kruskal-Wallis test on the individual variables).

To explore occurrence of biodiversity within the OTUs, specimens of all OTUs were then compared using a within-group PCA based on the correlation matrix. Individuals that on visual inspection of PCA plots were distinctly different from other specimens of the same OTU were checked in detail.

All localities were mapped, including data from published records, examined museum specimens and 60 localities from personal field tours (Figure 5-1); physiography, altitude, vegetation and climate of the localities were documented as well. Latitude of the localities was determined using Google Earth software (2010). Physiography and vegetation of the localities was determined using a Land Use Planning Map of Iran (Organization of Forests and Pastures of Iran, 1985) and the pertaining climate with the Climate Map of Iran (Khalili 1996).

5-3- Results

5-3-1- Construction of Operational Taxonomic Units (OTUs)

Based on the geology and geographical background of the northern and western mountains of Iran, *Natrix tessellata* specimens were grouped into three OTUs, including: the north OTU for northern Zagros, Azarbaijan and Alborz mountains, the middle OTU for the central Zagros and the southern OTU for the southern Zagros range (Figure 5-1). The Central Zagros mountain is the highest part of the Zagros chain and geographically separates the northern and southern Zagros from each other. The northern OTU contains specimens of Khorasan province, Azarbaijan province, seashores of Caspian sea and specimens from the Alborz mountains; the middle OTU contains specimens of central Zagros and Kermanshah province; and the south OTU contains specimens of the southern Zagros mountains which mainly originate from Fars province. The descriptive data of examined characters in each OTU have been presented in Table 5-1.

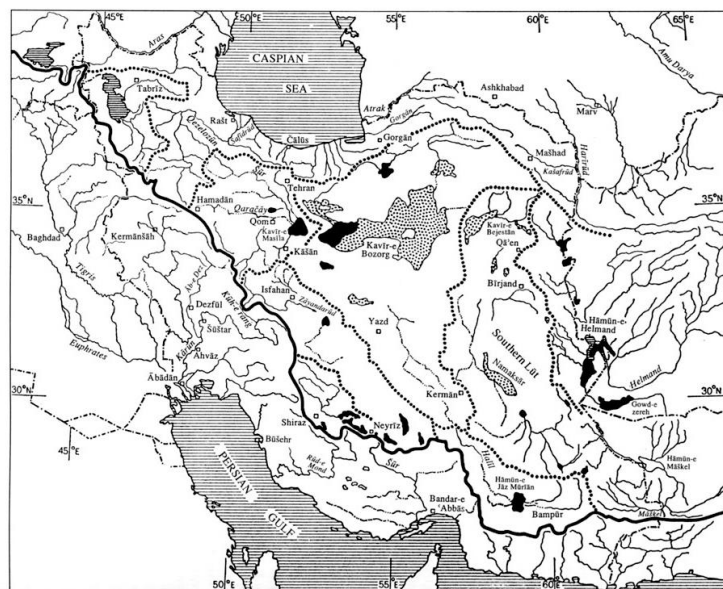
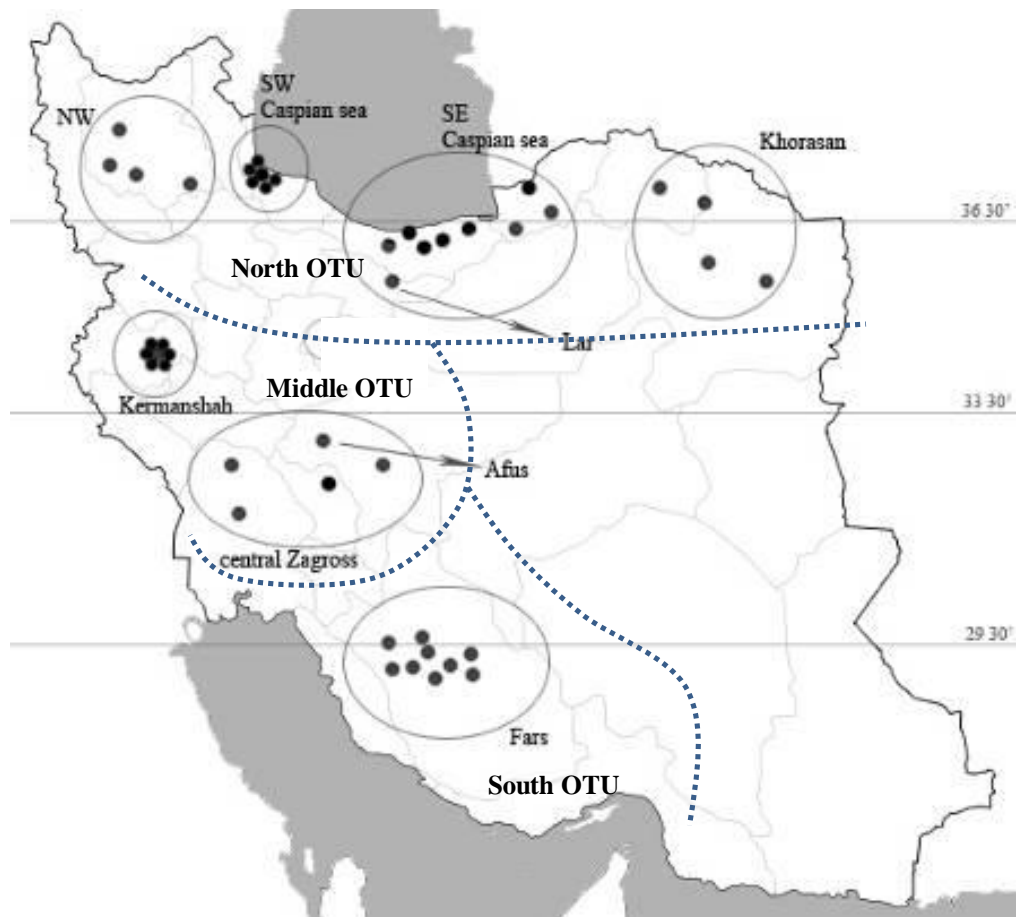


Figure 5-1. Above: map showing the localities of examined specimens of *Natrix tessellata* in Iran, as well as an indication of the north, middle and south OTUs, below: hydrographic map showing the main rivers of Iran (Fisher, 1968). *Natrix tessellata* inhabit semi-aquatic habitats.

Table 5-1. Descriptive statistic (above: mean \pm S.E., below: range) of morphological characters for male and female specimens of *Natrix tessellata* in Iran partitioned into geographic regions (OTUs). The ratio characters were multiplied by 100. Definition of characters are in Table 2-1.

<u>Males</u>			
OTUs	Northern	Central	Southern
Number	17	10	12
TL/TOL	20.61 \pm 0.29 18.65 - 22.95	21.74 \pm 0.56 19.23 - 24.43	19.78 \pm 0.3 18.56 - 22.31
HL/TOL	4.03 \pm 0.09 3.5 - 5.2	3.84 \pm 0.17 2.64 - 4.61	3.32 \pm 0.11 3.06 - 4.29
HL/TOL	2.2 \pm 0.07 1.86 - 2.99	2.028 \pm 0.07 1.79 - 2.54	1.94 \pm 0.04 1.72 - 2.39
Ven	171.29 \pm 1.05 165 - 179	172.3 \pm 1.07 167 - 176	175.08 \pm 1.26 170 - 185
Scd	64.47 \pm 1.13 55 - 72	66.4 \pm 1.42 58 - 73	62.92 \pm 0.81 59 - 67

<u>Females</u>			
OTUs	Northern	Central	Southern
Number	13	7	7
TL/TOL	21.71 \pm 0.3 19.73 - 23.24	20.84 \pm 0.72 17.39 - 22.86	20.84 \pm 0.67 18.58 - 23.57
HL/TOL	3.86 \pm 0.11 2.92 - 4.47	3.78 \pm 0.2 2.99 - 4.63	3.66 \pm 0.21 3 - 4.57
HL/TOL	2.19 \pm 0.14 1.68 - 3.69	2.02 \pm 0.12 1.58 - 2.44	2.13 \pm 0.07 1.85 - 2.35
Ven	172.92 \pm 1.42 163 - 179	173.86 \pm 1.79 167 - 182	173 \pm 1.35 168 - 177
Scd	68.69 \pm 1.42 60 - 78	67.86 \pm 3.07 50 - 73	66 \pm 2.28 56 - 72

5-3-2- Multivariate and univariate comparison of OTUs

The three OTUs were subjected to between-groups principal component analysis (PCA) based on the correlation matrix. The scatter plot resulting from the between groups PCA on the male specimens of *Natrix tessellata* (Figure 5-2) reveals that the southern OTU diverges from the north and middle OTUs mainly by snout vent length and number of ventral scales. In female specimens (Figure 5-3) a similar result is observed but not as clear as in male specimens. Principal components till the level of 95% of variation are in Table 5-2.

Table 5-2. Principal components until the level of 95% of variation in male and female specimens of *Natrix tessellata*.

Male		Female	
PC	% variance	PC	% variance
1	79.843	1	84.696
2	20.157	2	15.304

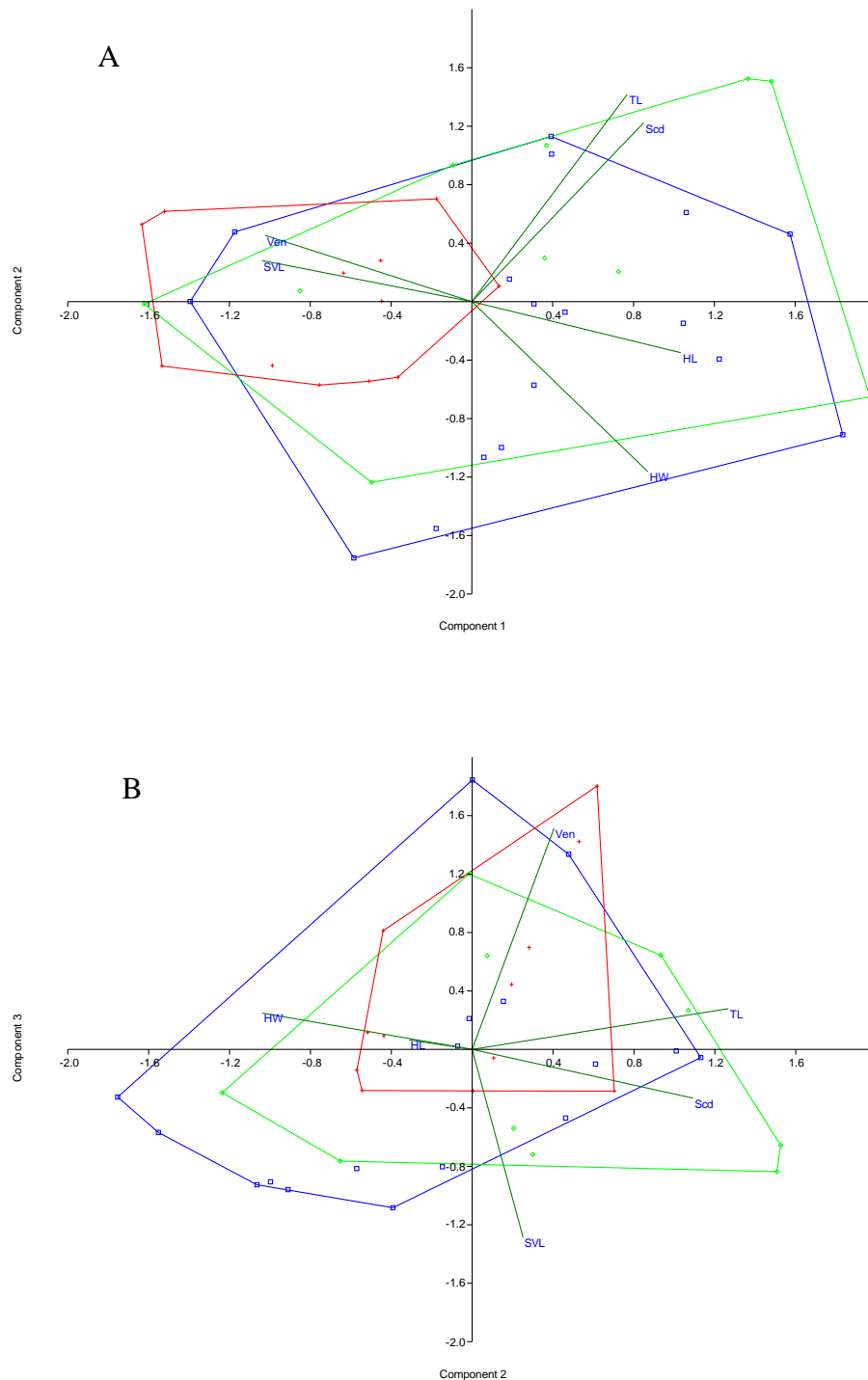


Figure 5-2. Scatter plot resulting from the between-group PCA showing a divergence of north (blue), middle (green) and south (red) OTUs in male specimens of *Natrix tessellata*. A: component 1 vs. 2; and B: component 2 vs. 3. All variables and their contribution (factor loading) in OTU divergence are superimposed on the plots.

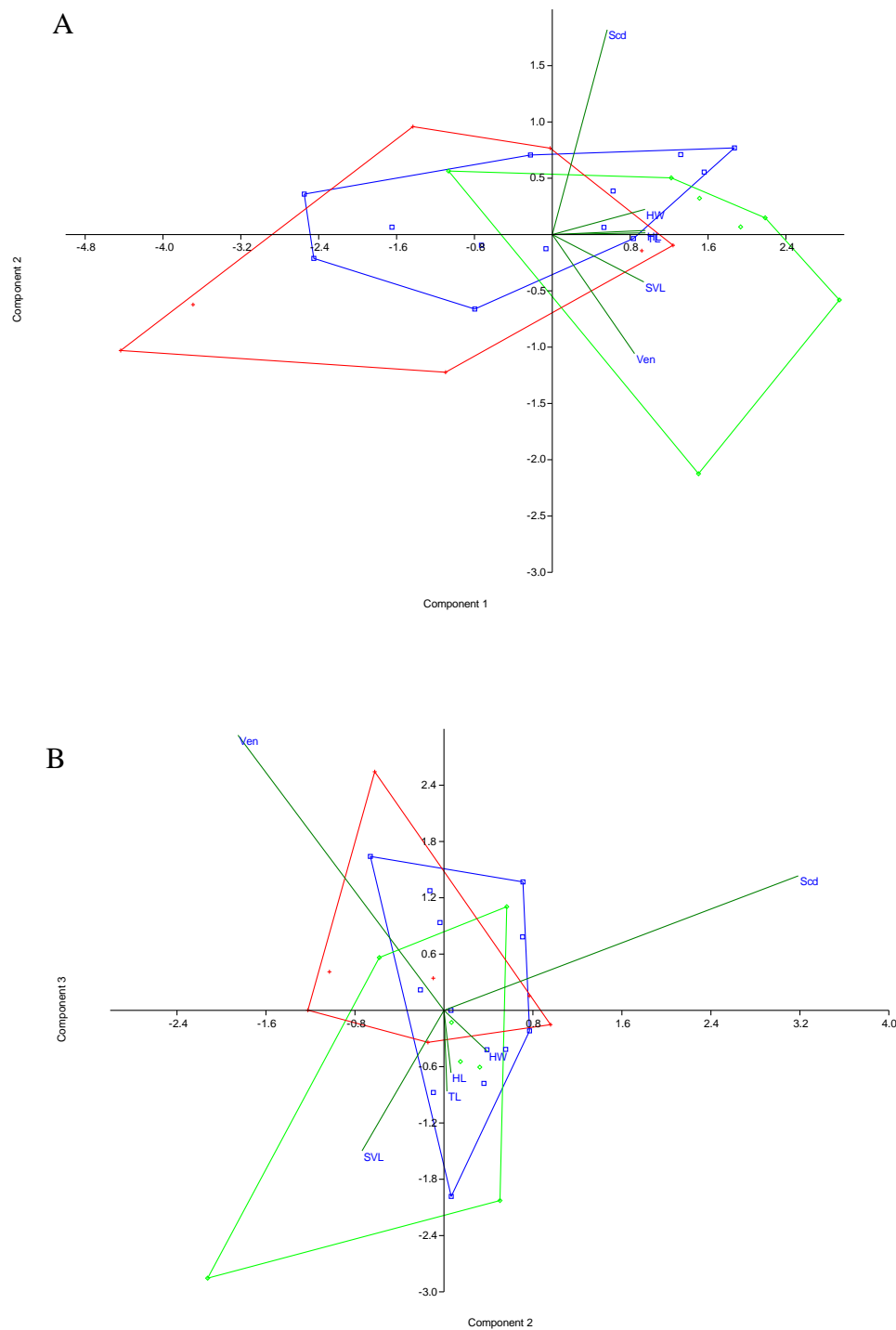


Figure 5-3. Scatter plot resulting from between-group PCA showing a divergence of north (blue), middle (green) and south (red) OTUs in female specimens of *Natrix tessellata*. A: component 1 vs. 2; and B: component 2 vs. 3. All variables and their contribution (factor loading) in OTU divergence are superimposed on the plots.

MANOVA reveals that males from the south OTU are significantly different from those of the north (table 5-3).

Table 5-3. Bonferroni-corrected p values, resulting from the multivariate analysis of variance (MANOVA) on male and female specimens of *Natrix tessellata*.

Male				female			
-	North	Middle	South	-	North	Middle	South
North	-	0.25	<0.05	North	-	0.23	0.25
Middle	0.25	-	0.03	Middle	0.23	-	0.10
South	<0.05	0.03	-	South	0.25	0.10	-

Table 5-4. Bonferroni-corrected p values, resulting from the one way non-parametric multivariate analysis of variance (NPMANOVA) in male and female specimens of *Natrix tessellata*.

Male				Female			
OTUs	North	Middle	South	OTUs	North	Middle	South
North	0	0.9964	0.6274	North	0	0.0434	0.8567
Middle	0.9964	0	0.5374	Middle	0.0434	0	0.0779
South	0.6274	0.5374	0	South	0.8567	0.0779	0

The ANCOVA and ANOVA with LSD post hoc pairwise test between male specimens of these three OTUs reveals that the southern OTU is significantly different from the northern in the tail length (TL), head length (HL), head width (HW) and number of ventral scales (Ven) (Table 5-5). In a detailed view, TL, HL and HW in the southern OTU are shorter than in the northern OTU; also, specimens from the southern OTU have a higher number of ventral scales than northern OTU (Figure 5-4). The three OTUs aren't significantly different in non-parametric characters (Table 5-4).

Table 5-5. Result of ANCOVA (for metric characters) and ANOVA (for meristic characters) with LSD post hoc pairwise test between the three OTUs on morphological characters of male specimens.

	OTU	OTU	Sig.
TL	Northern	Central	0.02
		Southern	0.16
	Central	Northern	0.02
		Southern	0.00
	Southern	Northern	0.16
		Central	0.00
HL	Northern	Central	0.25
		Southern	0.00
	Central	Northern	0.25
		Southern	0.00
	Southern	Northern	0.00
		Central	0.00
HW	Northern	Central	0.08
		Southern	0.00
	Central	Northern	0.08
		Southern	0.30
	Southern	Northern	0.00
		Middle	0.02
Ven	North	Middle	0.54
		South	0.02
	Middle	North	0.54
		South	0.12
	South	North	0.02
		Middle	0.12
Scd	North	Middle	0.25
		South	0.33
	Middle	North	0.25
		South	0.06
	South	North	0.33
		Middle	0.06

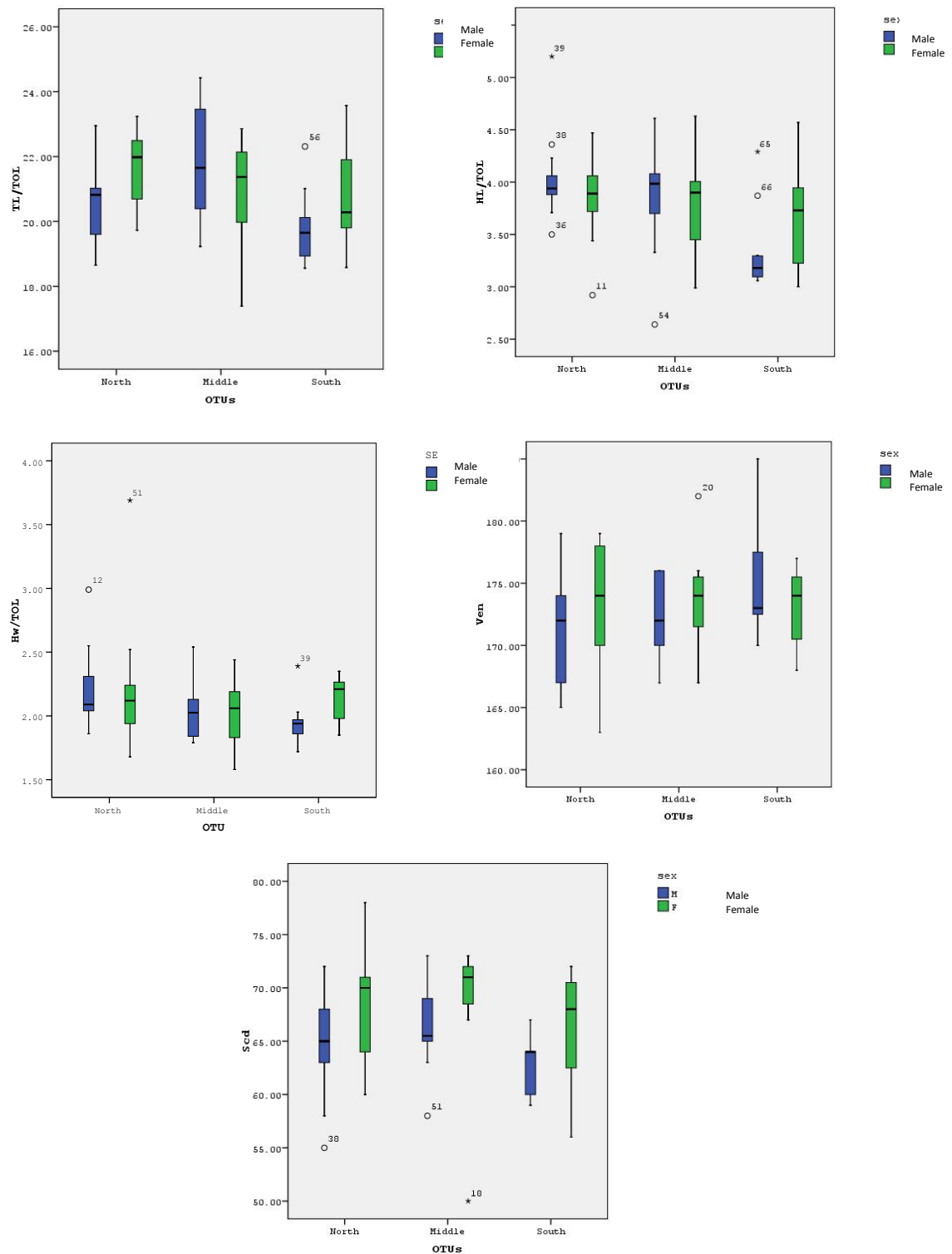


Figure 5-4. Box plots showing the minimum, first quartile, median, third quartile, and maximum of morphological characters throughout the OTUs. The ratio characters were multiplied by 100.

5-3-3- Intra-OTUs variation

All specimens of the three OTUs were pooled into a single within-group principal component analysis (PCA) based on the correlation matrix. The first three components in males and the first two in females cover 95% of total variation (Table 5-6). The scatter plots (Figure 5-5) show that PC1 is mainly explained by variation in size characters, and reflects a size PC, whereas PC2 appears to be mostly correlated with meristic variables. In PCA scatter plots, most of the individuals showed no extreme morphological difference with the rest of the specimens in the OTU. Detailed checking of two specimens with high morphological difference (showed by an arrow in figure 5-5 B and C) revealed that they are not geographically isolated. They are morphologically different from other specimens in the same locality due to the natural individual variation within the population.

Table 5-6 Principal components covering 95% of total variation in male and female specimens of *Natrix tessellata*.

PC	Eigenvalue	% variance	PC	Eigenvalue	% variance
1	165.39	90.29	1	194.72	94.03
2	8.42	4.6	2	6.77	3.27
3	5.36	2.93	3	3.35	1.62

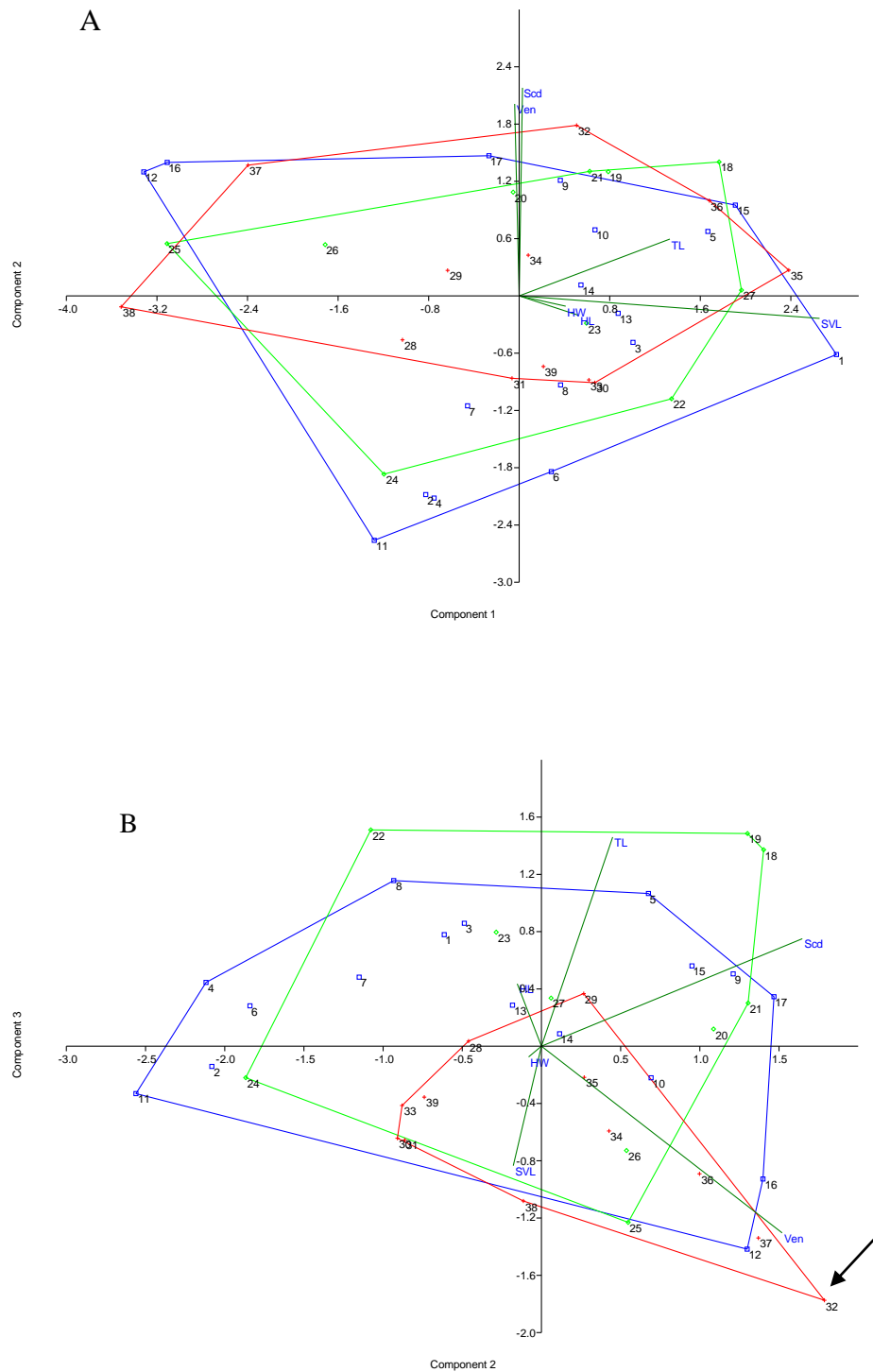


Figure 5-5. Scatter plot from the within-group PCA on all pooled data showing all individuals of north (blue), middle (green) and south (red) OTUs in male specimens of *Natrix tessellata*. A: male specimens, component 1 vs. 2, B: male specimens, component 2 vs. 3, C: female specimens component 1 vs. 2. Arrows indicate individuals with a high morphological difference. Continued on next page.

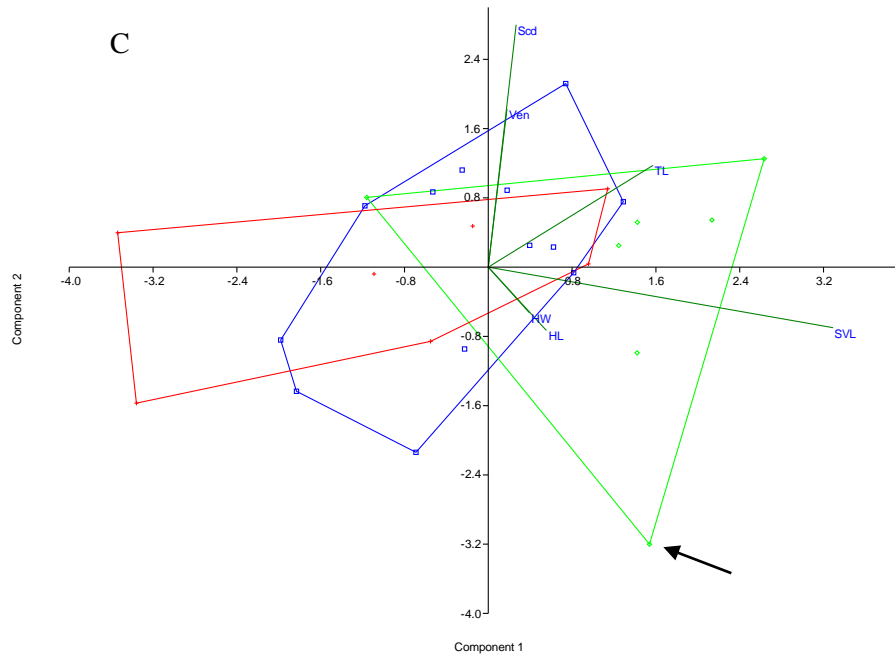


Figure 5-5. Continued. Caption in previous page.

5-3-4- Distribution

All known localities of *Natrix tessellata* are shown in Figure 5-6. The localities originate from field observations by the M. Rajabizadeh, localities of examined specimens in collections, a review of literature, especially Latifi (1991, 2000), and personal communications with other herpetologists in Iran. The southernmost known localities of *N. tessellata* in Iran reach up to the northern and central Fars province. Efforts for finding any locality of this snake species in the south of Fars province or in Bushehr and Bandar Abbas provinces in the vicinity of the Persian Gulf yielded no positive results, hence, its distribution in the southern branches of the Zagros mountains remains unclear. Since there is no confirmed record of this species in the southeastern Iran, the presence of this species in southeastern Iran is doubtful, even though Latifi (2000) reported *N. tessellata* from Sistan and Baluchestan provinces without exact localities. On the other hand, M. Rajabizadeh has received unconfirmed reports of the presence of *N. tessellata* in the Bahr Aseman Mountains in Kerman province which need further confirmation. In summary, the distribution of *N. tessellata* in Iran is presumably restricted to the Alborz and Azarbaijan mountains, as well as the northern, central and western parts of the Zagros Mountains and adjacent areas. The species is

widely distributed in the plains of northern Alborz that drain into the Caspian Sea. It colonized arid plains to the east of the Caspian Sea and west of the Zagros mountains but in the central plains of Iran it occurs only along a few freshwater rivers.

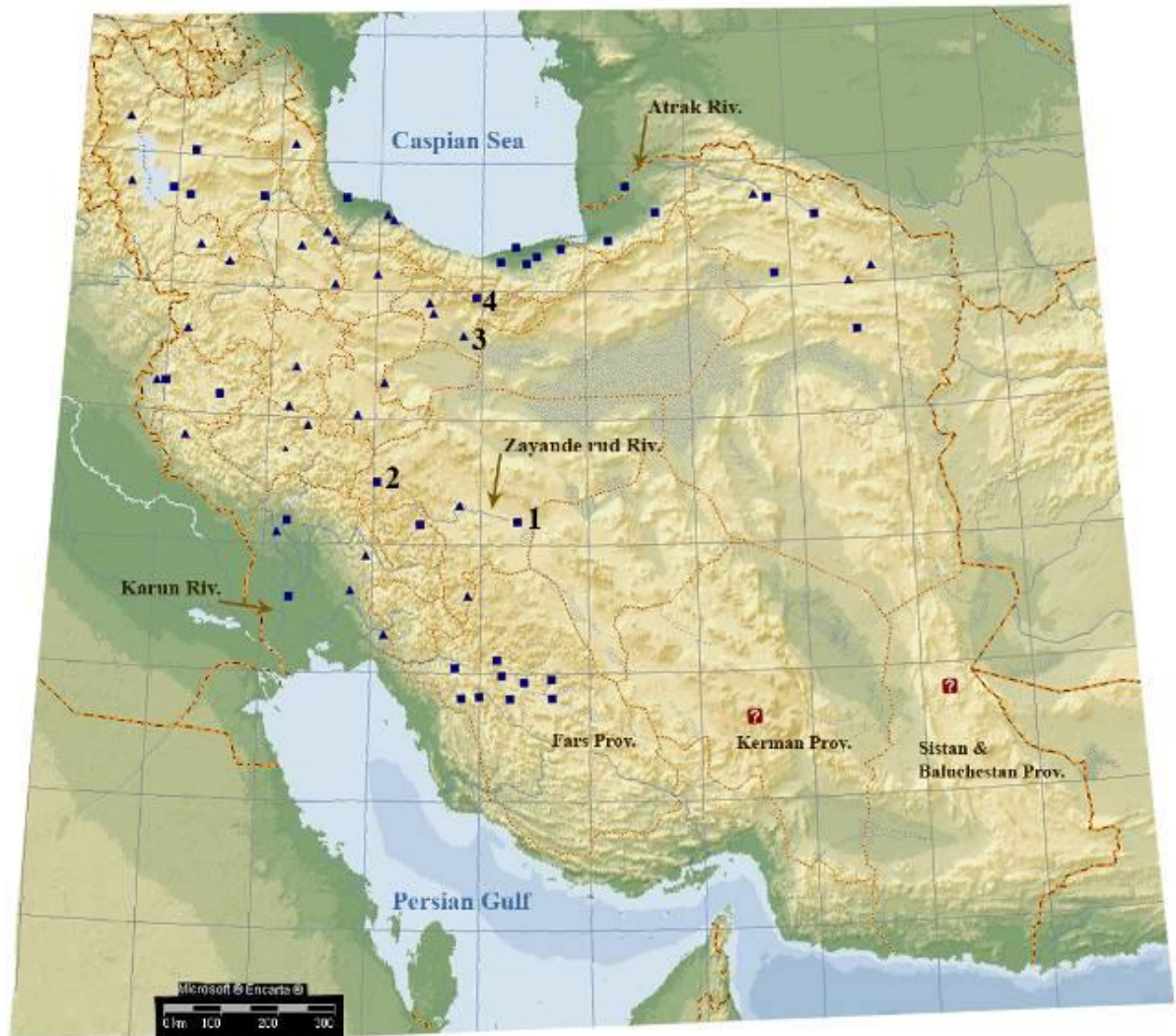


Figure 5-6. All known localities of *Natrix tessellata* in Iran. Quadrates represent localities from where specimens have been examined and used in the analysis. Triangles indicate localities reported in museum documents, literature or reliable personal communications with herpetologists from Iran. Question marks indicate the two localities of *N. tessellata* in southeastern Iran, mentioned by Latifi (2000, 1991), that require confirmation.

5-3-6- Habitat

To assess the habitat of *Natrix tessellata* in Iran, elevation, vegetation and climate of all the noted localities in Figure 5-6 were checked carefully and summarized. As a result, the habitat of *N. tessellata* in Iran can be classified into the following clusters:

- Lowlands and sea shores of the Caspian Sea: This area contains plains with elevations between 0–100 m and is restricted between the sea shore and the mountains. The climate is "Wet Caspian" and the original forest vegetation has mostly been altered into rice plantations. It is not clear whether this habitat alteration has had a positive or negative effect on the populations of *Natrix tessellata* in this region. But now, the species is abundant here and due to an abundance of surface water and associated prey, it is observed widely in this lowland.
- High elevated plains and mountainous habitat: This is the predominant habitat of *N. tessellata* in Iran. It starts at an elevation of 100 m on the northern slopes of the Alborz mountains. However, in the southern Alborz mountains, in the Azarbaijan and the Zagros mountains, equivalent lowland habitats are replaced by high mountain plains and mountains at about 1000 m elevation, as these mountains are open to the elevated central Iranian plains. In this habitat the species is observed normally near numerous streams and some rivers, artificial dams, pools and springs. Most of the habitats in the Alborz mountains consist of mountain slopes and valleys with a seasonal Mediterranean pasture vegetation, fed by spring rains. In the Azarbaijan and Zagros mountains the species also occurs on high elevation plains with low to high density pasture vegetation and mountain slopes with pasture vegetation, which in the Azerbaijan mountains changes to forests of *Juniperus* at higher elevations. Further south, in the Zagros mountains, the pastures change to *Pistacia* and *Amygdalus* vegetation with a Mediterranean climate with a few spring rain. The maximum elevation records for *N. tessellata* in this habitat type is from the Lar Valley in the Alborz mountains, where we found this species inhabiting a lake at the bottom of the valley (2500 m, see Figure 5-7, 5-8) and also a mountain brook in a side valley up to 2700 m (Figures. 5-8, 5-9). A comparably high locality of *N. tessellata* at 2500–2700 m is at the bottom of a mountain river valley in the Afus region of the Zagros mountains. This locality is a small artificial reservoir lake with a dam. Dice

snakes are abundant in the lake and manmade boulders around it. The lake is located at 2544 m and suitable habitat exist at another pond at 2635 m and mountain brooks higher up to 2700 m. Surrounding mountains rise up to 4000 m and are the type locality of two endemic lacertid lizards, *Iranolacerta zagrosica* (Rastgar-Pouyani and Nilson, 1998) and *Iranolacerta brandtii esfahanica* (Nilson, Rastegar-Pouyani, Rastegar-Pouyani and Andren, 2003). Both areas are characterized with a cold mountainous climate and an subalpine steppe with *Astragalus* sp. as the typical vegetation.

- Rivers in dry plains: *N. tessellata* can be found in dry plains along rivers in the eastern area of the Caspian Sea, western Zagros plains and central Iranian plains. East of the Caspian Sea this species has been recorded from the Atrak River, and in dry plains around the Alagol salt lake at an elevation of 35 m. In the western Zagros *N. tessellata* occurs along large rivers like Dez and Karun, which originate from the Zagros mountains and flow deeply into the Khuzestan plains, characterized by a hot dry desert climate, and finally reach the Persian Gulf. The species has been recorded from the western Zagros plains to around Ahvaz city at 15 m. In contrast, the species could not extend its distribution into the arid plains of central Iran, which lack large rivers, and thus has been recorded only from the margins of the central Iranian plains. Along the eastern versant of the Zagros mountains, the species has been recorded up to the end of Zayande Rud river in the Varzane area, just before the river reaches the Gavkhuni Lagoon, where it disappears in the desert. There, *N. tessellata* has been recorded at an elevation of 1400 m, in an area surrounded by desert and only 1 km from a sand dune. The river bed is flanked mainly by *Tamaryx* vegetation. Nearby sand dunes yield typical desert halophyte vegetation. South of Alborz, Latifi (1991) recorded a specimen from Varamin, just south of Tehran at the northern margin of the central Iranian plain at an elevation of 900 m. Both these marginal localities of *N. tessellata* have a semidesert climate. All the rivers that run from the Alborz and Zagros mountains into the central Iranian plain become dry or very salty outside the mountain valleys and it seems that these are the limiting factors for a further expansion of the dice snake into the dry plains. The species has not yet been reported from the coastal plain of the Persian Gulf in Khuzestan province.



Figure 5-7. Lar Valley in the Central Alborz mountains. Photo: M. GHASEMI



Figure 5-8. Locality in the Lar Valley, Central Alborz mountains, where two specimens of *N. tessellata* were found at 2700 m Photo: Benny Trapp



Figure 5-9. *Natrix tessellata* from the Lar Valley, Central Alborz mountains, feigning death and producing a deterring autohaemorrhagy. Photo: Benny Trapp.

5-4- Discussion

5-4-1- Distribution of *Natrix tessellata* in Iran

Even after detailed investigations in the course of this review, there is no report of *Natrix tessellata* from the plains along the Persian Gulf in southern Iran, except for an unconfirmed report in the Bushehr province (see above). Concerning the localities of the species in southeastern Iran, localities in the Kerman province need confirmation, as well as the localities noted by Latifi (2000) in Sistan and Baluchestan provinces, because most of those records originate from snake hunters. Although Latifi (2000) presented some exact localities for snakes of Iran, he usually didn't trust the statements of snake hunters. Consequently, most of his published localities are not precise and often only the province of the sampled specimens was reported and more information is not available. Hence, it remains unclear whether specimens of *N. tessellata* from Sistan and Baluchestan provinces examined by Latifi originate from that province or were transported there for commercial issues. In 2008, during a project of the Department of the Environment of Kerman province, M. Rajabizadeh examined a specimen of *Natrix natrix* collected by an amateur herpetologist from palm gardens around Bam City (29° 06' N, 58° 21' E), a city adjacent to the Lut Desert. Until further findings, we presume that this specimen was released there by humans,

hence, is not autochthonous. This leaves the localities in the Fars province as the confirmed southern range limit of *N. tessellata* in Iran, also representing the southernmost known distribution point of the species in its whole global range.

5-4-2- Habitat of *Natrix tessellata* in Iran

Considering the known localities of this species in northern Africa (Egypt) reveals that it is restricted to the large water course (Nile River), its immediate tributaries and its delta (Baha el Din, 2011). In contrast, the localities in Fars are related to rivers that run through high elevation plains and mountainous areas with natural lakes. The vegetation ranges from pasture to forest and the climate from Mediterranean to mountainous. For example, the Shiraz plain reaches an elevation of up to 1400 m and Arjan Lake is about 2000 m.

An important aspect of *Natrix tessellata* in Iran is its habitat in the mountains. Although this species uses rivers to even penetrate into arid plains, its occurrence in high elevated mountain rivers is a natural phenomenon, which can lead to local isolation of populations due to climatic fluctuations. In this study, the most elevated localities, recorded in Afus region in central Zagros (2700 m), and the Lar valley in central Alborz (2700 m) (Figure 5-7), yielded specimens that show morphological differences with *N. tessellata* in lower areas. An adult female specimen with a relatively unicolor light venter (CBSU C 881) was collected by M. Rajabizadeh and a team from Shiraz University Zoological Department, lead by Dr. H.R. Esmaeili, in Ghale Narenji (29° 29'N, 51° 44'E), connected to Parishan Lake at about 900 m elevation (Figure 5-10). It seems that this phenotype (tendency towards a unicolored venter) has a low frequency in some populations of *Natrix tessellata* in Fars province. This has not been reported ever from other populations of this snake in Iran, though it is known from populations throughout its range (Mebert 1993, 2011a).



Figure 5-10. Parishan Lake in Fars province. Photo: M. Rajabizadeh.

5-4-3- Geographic Variation of *Natrix tessellata* in Iran

Although in this chapter we tried to examine as many specimens of *N. tessellata* as possible, still the examined materials are not sufficient for a complete analysis of geographic variation of this species in Iran. Hence, the conclusions are to be seen as preliminary, yet related to the entire distribution of this species in the country.

The analysis show that male specimens of *Natrix tessellata* from southern Zagros mountains are significantly different from specimens of the northern mountains of Iran. The ANCOVA showed that TL, HL and HW are significantly shorter in specimens of southern Zagros mountains compare to specimens of the northern mountains of Iran. Southern Zagros specimens have significantly higher number of ventral scales than the specimens of the northern mountains. However, it is known that characters concerning head and body proportions, number of ventral and subcaudal scales vary greatly across regions in *N. tessellata* (Mebert 2011a; Brecko *et al.* 2011; Mebert, 1993; Gruschwitz *et al.* 1999; Madsen *et al.*, 1993) and are even subject to a pronounced microgeographic variation across 100–300 km (Mebert, 2011c; Mebert, 1996).

Underlying mechanisms for the geographic variation in the head length in Iranian *N. tessellata* is not known. Geographic variation of head length in *N. tessellata* has been

reported before (Mebert 1993), and was found to be significant for both sexes in southern Switzerland even across small distances of approximately 30–40 km (Mebert 1996). Moreover, Brecko *et al.* (2011) found differences in head size/shape between frog or fish eating specimens, viewing those diet differences as a potential factor governing geographic variation. Their analysis included many specimens from Iran, but without exact localities. Fish feeding *N. tessellata* had significantly narrower (relative longer) heads than dice snakes consuming frogs. They stated that it was not clear, whether the observed differences in head shape have a genetic basis or result from phenotypic plasticity.

The extensive geographic variation in a number of morphological characters observed in this study within Iran, and reported out of Iran (see Mebert, 2011a; Brecko *et al.* 2011; Gruschwitz *et al.* 1999; Mebert, 1993) suggests a more complex pattern and underlying causative factors. It remains to be seen whether a future analysis with a larger data set can corroborate such geographic variation for *N. tessellata* in Iran and find corresponding correlations with external factors.

5-4-4- Intra-OTU variation

The PCA on the pooled data of all OTUs didn't show specimens with great morphological divergence from the rest. This is concluded based on the examined morphological characters and the fact that most of the variation is variation in body and head shape and in the number of ventral and subcaudal scales.

Because of this, we looked at the habitat of *Natrix tessellata* in Iran. Although the distribution of this snake appears to be continuous through the northern and western mountains of Iran, altitude could restrict some isolated populations. Altitude isolation has been reported in many reptiles. A specimen from the Lar valley, central Alborz and one from Afus region, central Zagros, reflect the highest known altitude range of *Natrix tessellata* in Iran (up to 2700 m). Detailed morphological examination shows that the Afus specimen is quite unique in the dimensions of its head. It has a very short and wide head (low HL/HW of 1.29 in the Afus specimen vs. 1.99 in the middle OTU) and correspondingly low HL/TOI value of 2.94 vs. 3.64. As the observed overall variation falls within the normal morphological variation of central Zagros specimens and these differences are not supported by meristic characters, it is more likely that

this is an individual difference, which may be caused by innate or external factors, such as a damaged head bone, a disease, or a distortion of the head after preservation. The other distinct *N. tessellata* is a single male from the Lar valley (Figure 5-11). This specimen is different from the neighboring specimens from the northern OTU, mainly in the number of subcaudals (72 vs. a mean of 64.4 in the northern OTU) and mean tail length over total length (TL/TOI) (20.82 vs. 20.68), which indicates that this specimen exhibits a relatively higher number of subcaudals and correspondingly higher ratio of tail length over total length than other male specimens in the northern OTU. However, the difference of approximately 8 subcaudals to the mean of 64.4 subcaudals of the northern OTU is within the intra-population range of normally 10-15 subcaudals (max. range to even 26 subcaudals) found in various populations of *N. tessellata* in western Europe (Mebert, 1993).

5-4-5- Taxonomic comments

From a taxonomic point of view, the distinguishable variation in morphometric and meristic characters of *Natrix tessellata* in Iran across a latitudinal range from north to south suggests a potential subspecific differentiation. Detailed examination of the differences between the southern Zagros population (Fars) of *N. tessellata*, and the Alborz and northern Zagros populations shows that this difference is not so clear cut to allow us to treat the Fars population as a separate taxon at this time. But there are significant differences between populations of *N. tessellata* from Fars province (the southern OTU) and other populations of the species in the country (the northern and middle OTUs). A more detailed study, including a molecular comparison of populations, should be accomplished to clarify whether the Fars population deserves a special taxonomic status.

Guicking *et al* (2009, 2006) and Guicking and Joger (2011) in their study about the phylogeny and phylogeography of the genus *Natrix* and its species, included only limited



Figure 5-11. A male *Natrix tessellata* from the Lar valley in the central Alborz mountains. Photo: M. Rajabizadeh.

DNA of Iranian specimens, from Kermanshah province and the Lar valley. These were highly different from dice snakes outside of Iran but closely related to each other. This study brings further evidence for the distinct status of *N. tessellata* from Iran. However, we feel not confident to draw any taxonomic conclusions before further molecular studies shed light on the phylogeography of this species in Iran. However, from a global perspective, the Iranian *N. tessellata* studied by Guicking *et al.* (2011)

shows such a high genetic distance from all other *N. tessellata* populations that even the recognition of a separate species of Iranian dice snakes may be justified.

5-4-6- Biogeographic aspect

In order to explain the role of ecological and environmental factors that caused differentiation of the southern Zagros populations of *Natrix tessellata* (the southern OTU) from the Alborz and rest of the Zagros populations (the northern and middle OTUs), we should focus primarily on the biogeographic and geologic history of the southern Zagros region.

Guicking *et al.* (2009; 2006) and Guicking and Joger (2011) concluded that *N. tessellata* originated in southwest Asia and this evolution may have occurred during at least 5–6 Mya as a result of the varying and fluctuating environmental conditions associated with the deterioration of the climate at the end of the Miocene. Special focus on climate change phenomena in the Iranian plateau shows that during the glaciation period, the south of the Iranian plateau played an important role as a large refugial area for northern reptile species (Rastegar-Pouyani 2005, 2006). Recent studies show that although northern Iran was affected by glacier formation and temperature was significantly reduced during periods of glaciation, such effects of severely cold climate produced a less significant temperature reduction in southern Iran, including southern the Zagros (Klinsley, 2009; Ahmadi and Feiznia, 2006). Therefore, we suggest that numerous taxa of the herpetofauna from northern regions found a refugial area on the Iranian Plateau during periods of glaciation. After the end of each glaciation period, reptile species in the southern refugia moved again to the north and colonized vacant niches. However, our findings of significant morphological differences between northern and southern (Fars) Iranian *N. tessellata* preclude any conclusion that the glacial refugia of the northern populations were as far south as Fars province.

In reference to Guicking *et al.* (2009; 2006), we suggest that populations of *N. tessellata* in southern Zagros (Fars) represent one of the oldest populations of this species, which did not go extinct during periods of glaciation and that its morphological variations present today is the result from a long time of isolation affected by environmental factors of the southern Zagros. In contrast, the morphological similarity among the northern populations is the result of a recent and fast dispersal after the last glaciation (Guicking *et al.*, 2011).

Our preliminary data do not prove that Iranian *N. tessellata* are a monophyletic group with respect to non-Iranian dice snakes. It may turn out that southern Zagros (Fars) populations belong to an even older branch than those Iranian populations that have already been studied with molecular methods. Hence, it is paramount that further phylogenetic studies also include genetic data from populations of *N. tessellata* from the southern Zagros (Fars province), which may shed a new light on the taxonomy and biogeography of this species.

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CHAPTER 6

Taxonomy, Distribution and Geographic Variation of *Eirenis punctatolineatus* (Boettger, 1892) (Serpentes: Colubridae)

Modified from: Rajabizadeh, M., Schmidtler, J. F., Javanmardi, S., Rastegar-Pouyani, N. & H. R. Esmaili. (2012). Taxonomy, distribution and geographic variation of *Eirenis punctatolineatus* (Boettger, 1892) (Reptilia: Colubridae). *Amphibia-Reptilia*, 33(1): 69-82.

Abstract:

Distribution and geographic variation in *Eirenis punctatolineatus* are studied in the whole distribution range of the species. The results show that the species is distributed from central Turkey in the west, to the east, up to the northern Khorasan mountains in Iran and from central Armenia in the north, to the south, up to the adjacency of the sea shores of the Persian Gulf. A multivariate analysis revealed that the northern populations of *Eirenis punctatolineatus* (Turkey, Transcaucasia and northwestern Iran), and the southern populations (southern Zagros mountains and adjacent areas) are morphologically clearly distinct, but the species morphology is intermediate in the middle area of its range. A subspecific subdivision is suggested for the northern and southern populations. As a new combination, *Eirenis punctatolineatus condoni* (Boulenger, 1920), is proposed for the southern populations.

Key words: *Eirenis punctatolineatus*, distribution, geographic variation, taxonomy.

6-1- Introduction

Eirenis punctatolineatus is a member of the subgenus *Pediophis* Fitzinger, 1843, its nearest relatives being the levantine species *E. barani* Schmidtler, 1987 and *E. levantinus* Schmidtler, 1993 (see Nagy *et al.*, 2003).

Eirenis punctatolineatus originally was described by Boettger (1892) as a variety of *Eirenis modestus*, *Cyclophis (Eirenis) modestus* var. *punctatolineata*, based on the examination of one specimen from “Russisch Armenien” solely on the basis of color pattern. In pholidosis Boettger (1892) did not see any difference with the typical *Cyclophis (Eirenis) modesta*. *Cyclophis modesta* var. *punctatolineata* was described as having 170 ventrals and 66 subcaudals. In 1898, Boettger corrected the newly described taxon into *Contia collaris* var. *punctatolineata* adding the collection number (8261,1a) and a more exact origin of the specimen (“Erh. 1890 von der Reise Dr. Jean Valentin’s in den Karabagh”). Subsequent herpetologists (Nikolsky, 1916; Rostombekov, 1928) believed this taxon to be a subspecies of *Contia modesta* and finally Chernov (1939) raised it up to full species rank, under the name *Contia punctatolineata*.

The genus *Contia* of Boulenger (1894) was essentially a catch-all for various small snakes resembling each other in external characters. In 1914 Barbour proposed, presumably on geographic grounds, to use the name *Eirenis* Jan, 1863 for Old world (Africa, Asia, Europa) species (see also Stickel, 1951).

Although *Eirenis punctatolineatus* has a wide distribution range compared to other species in the genus *Eirenis*, few data are available on its exact distribution. In various publications, the distribution of the species has been noted as southern Armenia, Nakhichevan, southeastern Azerbaijan, eastern Turkey, and western Iran, (Ananjeva *et al.*, 2004; Szczerbak, 2003; Latifi, 2000; Terent év and Chernov, 1949). On the other hand, geographic variation of the species has been little studied (Schmidtler and Eiselt 1991).

Especially because of this lack of knowledge, several species and subspecies have been described by various herpetologists since 1892, now considered as synonyms of *Eirenis punctatolineatus*.

***Zamenis bornmüllerorum* Werner, 1903:** The description of this species was based on the examination of one specimen from “Elburs (=Alborz) mountain” “am Keredscli (supposedly Karaj)-Ufer”, Iran, at an elevation of 2100 m. Based on the original description, the specimen has one preocular, two postoculars, 1+2 temporals, 7 supralabials, 17 dorsals, 170 ventrals and 65 pairs of subcaudals. The anterior part of the dorsum displays two narrow transverse bands, followed by dark brown spots that change into dorsal longitudinal stripes toward the posterior part of the body (Spengel, 1904).

***Contia schelkovnikovi* Nikolsky, 1909:** Nikolsky (1909) described *Contia schelkovnikovi* based on the examination of one specimen from a dry slope near Dzhi village in the Lenkoran region (Azerbaijan Republic). *Contia schelkovnikovi* has 153 ventrals and 76 subcaudals based on its description.

***Contia condoni* Boulenger, 1920:** This species description was based on a couple of specimens collected from Shiraz (center of Fars province, Iran). Based on the original description, this species has one preocular, an undivided nasal, one small loreal, two postoculars, 1+2 temporals, 17 smooth dorsal scales, 169 - 173 ventrals, and 69 - 75 subcaudals. The dorsal pattern consists of small blackish spots forming narrow crossbars, continuous or interrupted and alternating on the back (Boulenger, 1920).

***Eirenis iranica* Schmidt, 1939:** This species was described based on a single adult male collected in 1934 from Tirak Mart mountain (now: Bibi Shahrbanu mountain) near Rayy (now: Rey), south of Tehran, Iran. Based on the original description, this specimen has one preocular, an undivided nasal that extended to the preocular, two postoculars, 1+2 temporals, 17 smooth dorsal scales, 159 ventrals and 76 subcaudals; without a color pattern except for two irregular small dark spots on the sides of the neck (Schmidt, 1939).

***Eirenis punctatolineatus kumerloevae* Eiselt, 1970:** This subspecies was described based on a single specimen from Akdamar Island in Lake Van, Turkey. It was distinguished from the nominal subspecies by a black dorsal coloration with scattered light spots. Franzen and Sigg (1989) raised questions about the validity of this subspecies, as only 11 of 22 specimens from Akdamar Island examined by them showed the black dorsal color. Also, molecular studies rejected validity of this subspecies (Nagy *et al.*, 2003).

In the current study, with the examination of different populations, morphological variation in relation to the distribution range of *Eirenis punctatolineatus* has been studied.

6-2- Materials and Methods

Based on extensive field expeditions and collection examinations, a total number of 44 specimens of *Eirenis punctatolineatus* including 23 males and 21 females were examined. Most of the Iranian specimens originate from ICSTZM, RUZM, ZMGU, CBSU, MMTT, DHZC, ZSM in Munich, Germany. The specimens of Armenia were borrowed from ZMMU.

Specimens were examined for 10 morphological characters including: six metric characters (SVL, TL, HL, HW, SL, SW), two meristic characters (Ven, Scd), eight binary and categorical characters (Pre, Inf, L.P.A.chin, Bet.8.Inf, Chin.Pre, P.Chin.Con, SBP, DP) (see Table 2-1 for definitions). Because patterns of sexual dimorphism in the number of ventral and subcaudal scales had been reported in various populations of *E. punctatolineatus*, separate analyses were carried out for males and females. For the descriptive analysis and graphic presentation of the metric data, ratios were used: $TL/TOL \times 100$, $HL/TOL \times 100$, $HW/TOL \times 100$, $SL/TOL \times 100$ and $SW/TOL \times 100$ (see Table 2-1 for definitions).

E. punctatolineatus is distributed across a wide area, and the examined specimens are geographically scattered. Hence, for analysis of geographic variation, specimens have been grouped into three geographic OTUs based on distance and geographic barriers. These OTUs include: northern OTU for the specimens of the northern mountains of Iran, central OTU for the specimens of the central Zagros and southern OTU for the specimens of the southern Zagros (Figure 6-1). To reveal character patterns of the different OTUs, descriptive statistical parameters including minimum, maximum, mean and standard error (S.E.) were calculated. The following analyses were done on the OTUs.

To investigate if geographical difference between OTUs is coupled to morphological difference, using metric and meristic characters, all OTUs were compared by between-groups principal component analyses (BG-PCA) based on the correlation matrix. Morphological characters that contribute to a separation of the OTUs in the PCA could

then be identified. Principal components were checked up to the level of 95% of total variation. To test whether the OTUs were significantly different, a MANOVA was performed, using characters that were normally distributed and showed a homogeneous variance (tested by Levene's test of homogeneity). For significantly different OTUs, discriminating characters between OTUs were identified using an analysis of covariance (ANCOVA) for metric characters (total length as covariate) and analysis of variance (ANOVA) for meristic characters. For the non-parametric characters analogous parametric tests were run to identify those variables that allowed to discriminate between OTUs (npMANOVA on combined variables followed by a Kruskal-Wallis test on the individual variables).

All available localities of *Eirenis punctatolineatus* examined in this study, present in the collections or reported in the literature, were mapped (Figure 6-1). The coordinates of the localities were obtained using Google Earth software (2010). More information on the localities and the examined specimens has been presented in appendix 1.

6-3- Results

6-3-1- Color and pattern

Two distinct color patterns distinguished in the northern OTU and some specimens of the central OTU, the typical pattern occurs, with posterior, dorsal, dark crossbars, interrupted on the dorsal midline. These crossbars are four scales long and 1 to 1/2 scale wide, separated by 1/2 to 2 scales from the next crossbar. Posteriorly, the crossbars become reduced to form dark spots and then progressively change to longitudinal, continuous or interrupted dorsal, parallel lines at 1/2 to 2/3 of the total length (Figure 6-2A). A slightly deviating pattern is found in some specimens, especially in specimens of Armenia, starting in the anterior part of the body with square-shaped dark spots (each crossbar is about two scales in length and two scales in width, so that they appear as square-shaped dorsal blotches) (Figure 6-2B).

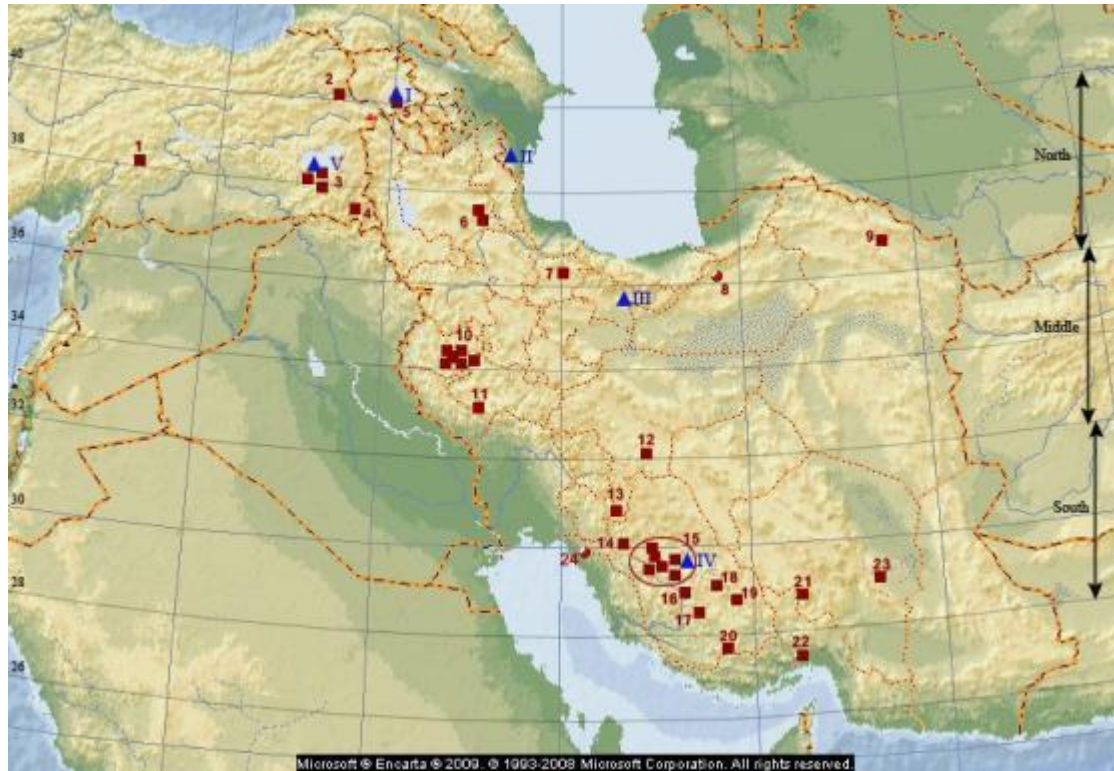


Figure 6-1. Distribution of *Eirenis punctatolineatus*, indicating the grouping of the specimens localities into three OTUs. North OTU: specimens between latitude 36°-40° N and more. Central OTU: specimens between latitude 32°-36° N. South OTU: specimens below and between latitude 28°-32° N. I: type locality of *Cyclophis modestus* var. *punctatolineata* Boettger, 1892 (given as “Russisch Armenien”). II: type locality of *Contia schelkovnikovi* Nikolsky, 1909. III: type locality of *Eirenis iranica* Schmidt, 1939. IV: Type locality of *Contia condoni* Boulenger, 1920. V: type locality of *Eirenis punctatolineatus kumerloevei* Eiselt, 1970. The type locality of *Zamenis bornmüllerorum* Werner, 1903 is not clear but supposedly is near to the type locality of *Eirenis iranica*.

A different pattern was observed in some middle, and especially southern OTU specimens, consisting of narrower dorsal crossbars, 0.5 – 1 scale wide, separated by 1 – 1.5 scales, which disappear at about the posterior 1/4 of the total length or progressively on the tail (Figure 6-2C). In fully grown adults, the dorsal crossbars become paler and more interrupted by light scales. In some specimens, especially those of the Kerman region, dorsal crossbars are about one scale wide and separated by one interval scale, so the pattern is denser and more or less similar to that of *Platyceps rhodorachis*. In the hind body, a color pattern is absent in these specimens. (Figure 6-2D).

Adult specimens without any dorsal pattern could be observed in all OTUs (Figure 6-2E).

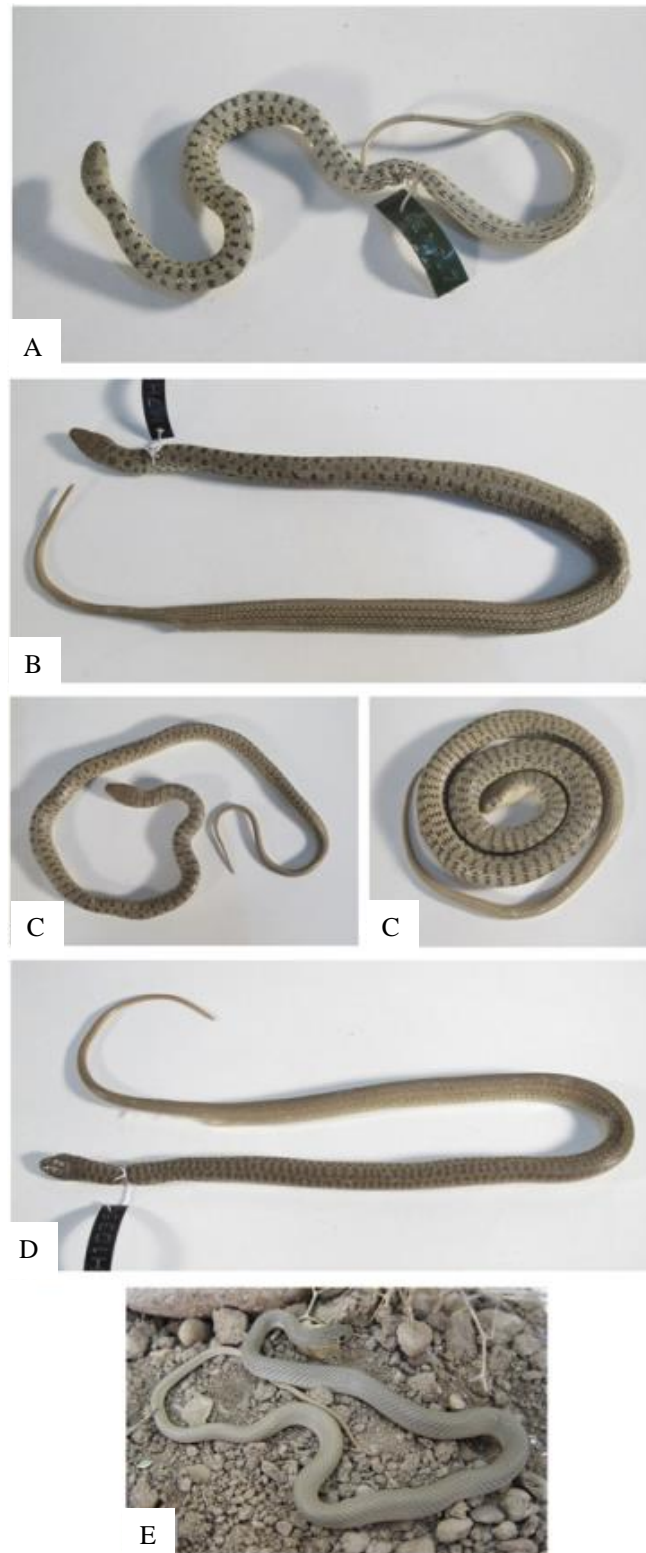


Figure 6-2. Different patterns in *Eirenis punctatolineatus*. A and B: Color pattern in specimens of the north and the central OTUs. C: Color pattern in specimens of the south and central OTUs. D: Color pattern in a specimen of Kerman region. E: A patternless specimen.

6-3-2- Construction of Operational Taxonomic Units (OTUs)

Based on the geology and geographical background of the northern and western mountains of Iran, *Eirenis punctatolineatus* specimens were grouped into three OTUs, including: north OTU for the northern Zagros, Azerbaijan and Alborz mountains, central OTU for the central Zagros and south OTU for the southern Zagros range (Figure 6-1). The Central Zagros mountain is the highest part of the Zagros chain and geographically separates the northern and the southern Zagros from each other. The South OTU refers to all the specimens distributed below and between latitude of 28°-32° N, the middle OTU refers to specimens distributed at a latitude of 32°-36° N, the north OTU refers to specimens distributed between the latitude of 36°-40° N and above 40° N.

Since *Eirenis punctatolineatus kumerloevae* from Akdamar Island, Lake Van is no longer regarded as valid here, the single specimen from there was included into the northern OTU, together with the other eastern Anatolian specimens.

The descriptive data of examined characters in each OTU are presented in Table 6-1. The data of different sexes have been presented independently.

6-3-3- Multivariate and univariate comparison of OTUs

The three OTUs were subjected to between-groups principal component analysis (PCA) based on the correlation matrix. The scatter plot resulting from the between-groups PCA on the male specimens of *Eirenis punctatolineatus* (Figure 6-3 A) reveals that the OTUs diverge from each other mainly by tail length and number of subcaudal scales. In female specimens (Figure 6-3 B) a similar result is observed. Principal components up to the level of 95% of variation are cited in Table 6-2.

Table 6-2. Principal components up to the level of 95% of variation in male and female specimens of *Eirenis punctatolineatus*.

Male		Female	
PC	% variance	PC	% variance
1	67.57	1	82.06
2	32.42	2	17.93

Table 6-1. Descriptive statistic (above: mean \pm S.E., below: range) of morphometric characters including Minimum, Maximum, Mean, and Standard Error in the northern, central and southern OTUs of *Eirenis punctatolineatus*. The ratio characters were multiplied by 100.

	Males		
	Northern	central	Southern
	10	6	10
TL/TOL	24.74 \pm 0.44 21.86 - 26.4	27.64 \pm 0.99 25.09 - 31.38	28.65 \pm 0.35 26.65 - 30.7
HL/TOL	4.12 \pm 0.19 3.36 - 5.43	3.77 \pm 0.12 3.45 - 4.13	3.81 \pm 0.11 3.24 - 4.39
HW/TOL	2.44 \pm 0.1 2.06 - 3.21	1.96 \pm 0.16 1.23 - 2.46	2.24 \pm 0.07 1.91 - 2.59
SL/TOL	1.29 \pm 0.08 1.09 - 1.82	1.1 \pm 0.04 0.96 - 1.21	1.4 \pm 0.09 0.94 - 1.7
SW/TOL	1.59 \pm 0.08 1.32 - 2.22	1.44 \pm 0.09 1.23 - 1.86	1.42 \pm 0.04 1.23 - 1.55
Ven	159.4 \pm 0.54 157 - 162	158 \pm 1.73 153 - 163	160.1 \pm 2.37 150 - 176
Scd	72.3 \pm 1.25 63 - 77	78.83 \pm 2.55 70 - 88	85 \pm 1.26 80 - 93

	Females		
	Northern	Central	Southern
	7	5	8
TL/TOL	22 \pm 0.36 20.99 - 23.17	25.71 \pm 0.61 24.53 - 27.56	25.92 \pm 0.51 23.53 - 27.73
HL/TOL	3.88 \pm 0.09 3.62 - 4.26	3.9 \pm 0.18 3.41 - 4.5	3.35 \pm 0.14 2.87 - 4.11
HW/TOL	2.31 \pm 0.08 2.1 - 2.76	2.29 \pm 0.09 2.15 - 2.61	2.06 \pm 0.1 1.68 - 2.6
SL/TOL	1.17 \pm 0.1 0.73 - 1.59	1.2 \pm 0.08 0.94 - 1.38	1.25 \pm 0.12 0.93 - 1.94
SW/TOL	1.49 \pm 0.03 1.38 - 1.62	1.45 \pm 0.08 1.24 - 1.7	1.3 \pm 0.09 1.04 - 1.82
Ven	165.7 \pm 2.65 155 - 175	165.2 \pm 2.37 157 - 170	168.5 \pm 2.34 159 - 176
Scd	64.6 \pm 1	69.8 \pm 0.8	77.4 \pm 1.93

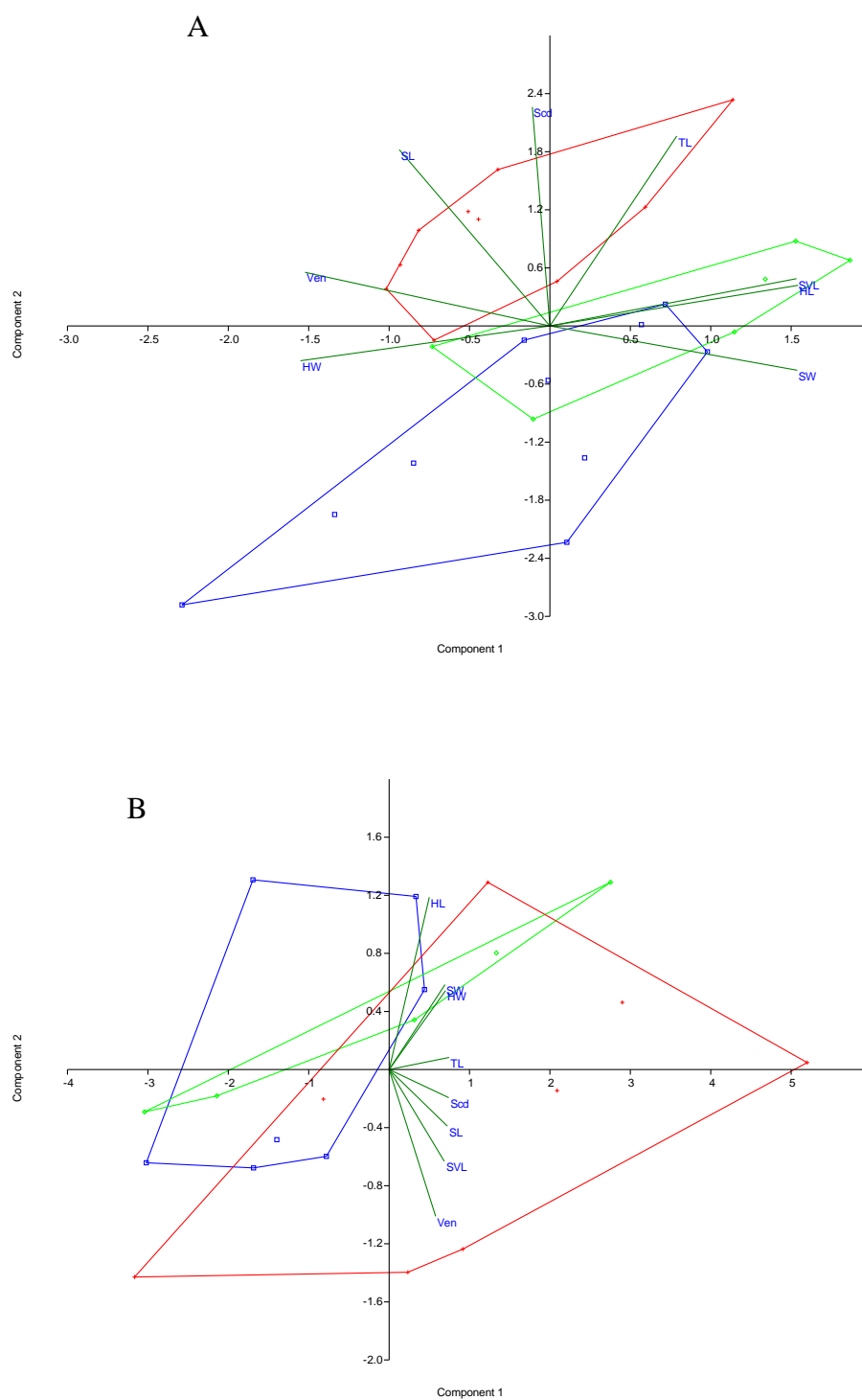


Figure 6-3. Scatter plot resulting from the between-groups PCA showing divergence of north (blue), center (green) and south (red) OTUs in male (A) and female (B) specimens of *Eirenis punctatolineatus*.

A MANOVA reveals that males and females from the south OTU are significantly different from those of the north (Table 6-3). The ANCOVA and ANOVA with LSD post hoc pairwise test between these three OTUs reveals that the south OTU is significantly different from the north OTU in the tail length (TL) and number of subcaudal scales (Scd) in both sexes (Table 6-4). Head length (HL) in males and snout width (SW) in female specimens are significantly different between the south and north OTUs too. In a detailed view, TL in the south OTU is higher than in the north OTU, but HL and SW in the south OTU are lower. The number of subcaudal scales in the south OTU is higher than in the north OTU (Figure 6-4).

Table 6-3. Bonferroni correcting p value, resulting from the multivariate analysis of variance (MANOVA) on male and female specimens of *Eirenis punctatolineatus*.

Male				female			
	North	Center	South		North	Center	South
North	-	0.13	<0.05	North	-	0.59	<0.05
Center	0.13	-	0.05	Center	0.59	-	0.09
South	<0.05	0.05	-	South	<0.05	0.09	-

Table 6-4. Result of ANCOVA (for metric characters) and ANOVA (for meristic characters) with LSD post hoc pairwise test between the OTUs on morphological characters of male and female specimens.

	OTU	OTU	Males	Females
			Sig.	Sig.
TL	Northern	central	0.00	0.00
		Southern	0.00	0.00
	central	Northern	0.00	0.00
		Southern	0.17	0.71
	Southern	Northern	0.00	0.00
		central	0.17	0.71
HL	Northern	central	0.55	0.54
		Southern	0.22	0.00
	central	Northern	0.55	0.54
		Southern	0.65	0.00
	Southern	Northern	0.22	0.00
		central	0.65	0.00
HW	Northern	central	0.01	0.76
		Southern	0.31	0.10
	central	Northern	0.01	0.76
		Southern	0.06	0.07
	Southern	Northern	0.31	0.10
		central	0.06	0.07
SL	Northern	central	0.36	0.87
		Southern	0.14	0.49
	central	Northern	0.36	0.87
		Southern	0.03	0.63
	Southern	Northern	0.14	0.49
		central	0.03	0.63
SW	Northern	central	0.60	0.83
		Southern	0.13	0.04
	central	Northern	0.60	0.83
		Southern	0.42	0.07
	Southern	Northern	0.13	0.04
		central	0.42	0.07
Ven	Northern	central	0.61	0.89
		Southern	0.77	0.42
	central	Northern	0.61	0.89
		Southern	0.44	0.38
	Southern	Northern	0.77	0.42
		central	0.44	0.38
Scd	Northern	central	0.01	0.04
		Southern	0.00	0.00
	central	Northern	0.01	0.04
		Southern	0.02	0.00
	Southern	Northern	0.00	0.00
		central	0.02	0.00

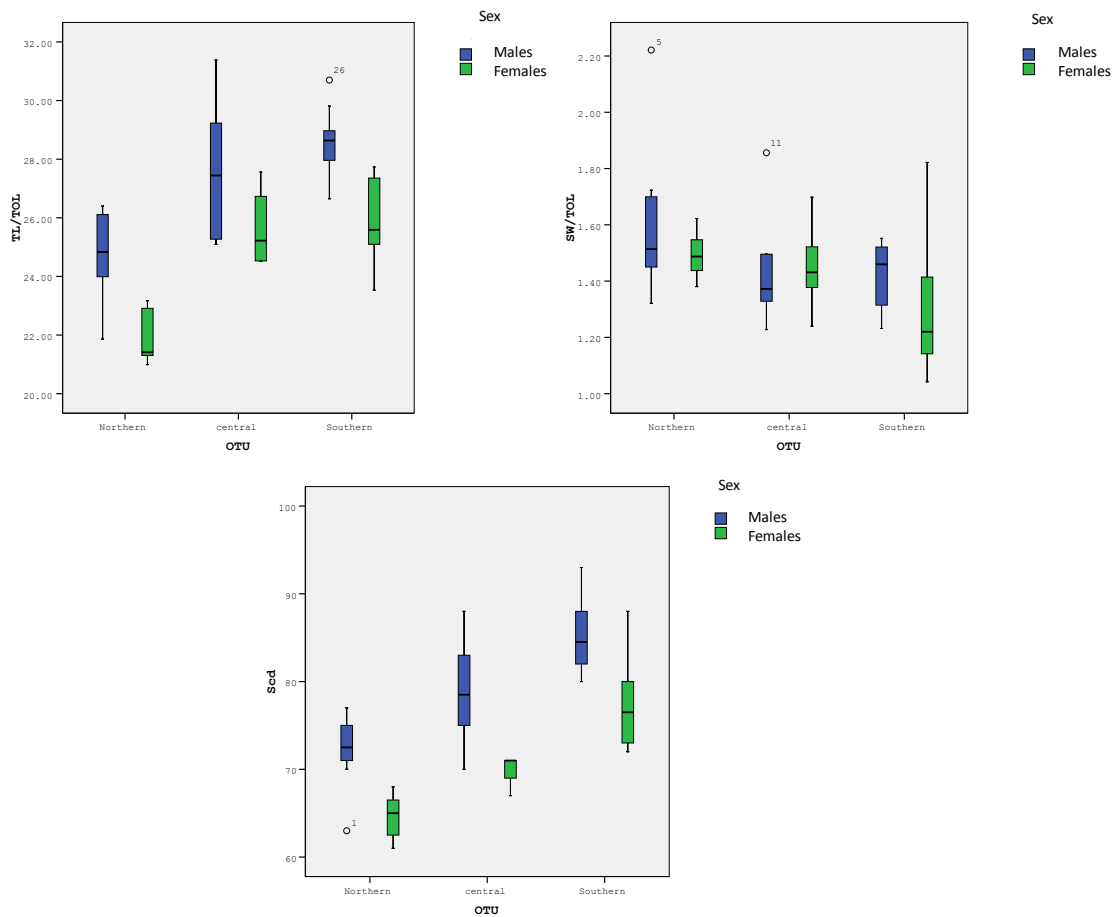


Figure 6-4. Box plots showing the minimum, first quartile, median, third quartile, and maximum of the significant morphological characters across the north, central and south OTUs. The ratio characters were multiplied by 100.

All three OTUs in females, and the northern and southern OTUs in males are significantly different in non-parametric characters (Table 6-5). The Kruskal-Wallis analysis reveals that the number of preventral scales (pre), the ratio of length of the posterior chin shield to the anterior one (L.P.A.Chin), the number of gular scales between the 8th infralabials (Bet.8.Inf), connection or separation of the posterior chin shields (P.Chin.Con), the scales bordering the parietals (SBP) and the dorsal color pattern (DP) are significantly different in males in the northern and southern OTUs. The number of preventral scales (pre), the number of gular scales between the posterior chin shield and the first preventral (Chin.Pre), the scales bordering the parietals (SBP) and the dorsal color pattern (DP) are significantly different in females all OTUs (Table 6-6).

Table 6-5. Bonferroni-corrected p value, resulting from the one way non-parametric multivariate analysis of variance (npMANOVA) in male and female specimen of *Eirenis punctatolineatus*.

Male				Female			
	North	Center	South		North	Center	South
North	-	<0.05	<0.05	North	-	0.04	<0.05
Center	<0.05	-	0.03	Center	0.04	-	0.07
South	<0.05	0.03	-	South	<0.05	0.07	-

Table 6-6. p value, resulting from the Kruskal-Wallis analysis on non-parametric characters in male and female specimen of *Eirenis punctatolineatus*.

		Pr	Inf	L.P.A.Chin	Bet.8.Inf	Chin.Pre	P.Chin.Con	SBP	DP
Males	Sig.	0.01	0.35	0.02	0.02	0.45	0.16	0.01	0.00
Females	Sig.	0.04	0.46	0.82	0.11	0.05	0.39	0.01	0.00

6-4- Discussion

6-4-1- Distribution

The results of this study give a more precise distribution range for *Eirenis punctatolineatus* we can summarize the distribution of the species as follows: central-eastern Turkey, central and southern Armenia, southern Azerbaijan, Nakhichevan, and northern and western Iran through the Elburz and Zagros Mountain chains and adjacent mountains of central Iran. In Iran, it occurs from eastern to northern Khorasan and south to the mountains adjacent to the sea shores of the Persian Gulf. Since the species is distributed close to border of Iraq in both Iran and Turkey, it is supposedly distributed in northern and northwestern Iraq too, adjacent to the distribution of the species in Turkey and Iran.

The species occurs in hills and mountain sides from about 200 m asl, adjacent to the northwestern Persian Gulf sea shores (locality number 24 in Figure 6-1) to more than

2000 m in eastern Turkey and northwestern Iran. It does not penetrate to arid lowlands like Khuzistan or into humid lowlands, like the southern regions of the Caspian Sea. The species is also absent from the high arid plains of central Iran.

6-4-2- Taxonomical and geographical variations

The analysis indicate that the northern and southern OTUs differ significantly in the tail length (TI), and number of subcaudal scales (Scd) in both sexes; also, these two OTUs differ from each other in the number of preventral scales (pre), number of scales bordering parietals (SBP) and shape of their dorsal color pattern in both sexes.

In morphometric and meristic characters, the specimens of the central OTU display an intermediate range of the characters between the northern and southern OTUs.

Based on these data, we propose that the southern OTU can be separated from the northern OTU at the subspecies level. Both subspecies occur in the transitional middle OTU. Since Boulenger (1920) used the name *Contia condoni* for describing a new species based on a couple of *Eirenis* specimens collected from around Shiraz, we use this name to refer to the southern subspecies of *Eirenis punctatolineatus*.

6-4-3- *Eirenis (Pediophis) punctatolineatus punctatolineatus* (Boettger, 1892)

Cyclophis modestus var. *punctatolineata* Boettger, 1892

Contia collaris var. *punctatolineata* Boettger, 1898

Zamenis bornmüllerorum Werner, 1903

Contia schelkovnikovi Nikolsky, 1909

Contia punctatolineata Chernov, 1939

Eirenis iranica Schmidt, 1939

Eirenis punctatolineatus kumerloevei Eiselt, 1970

Terra typica: “Russisch Armenien” (approximately the current Republic of Armenia) (Boettger, 1892 in Boettger, 1898)

Diagnosis: *Eirenis punctatolineatus punctatolineatus* is characterised by a combination of morphometric, meristic and color pattern characters, especially by the number of subcaudal scales, 63 – 77 ($X= 72.09$) in males and 61 – 68 ($X= 64.67$) in

females and the ratio of tail length over total length being 21.86 – 26.40 ($X = 24.66$) in males and 20.99 – 23.17 ($X = 22.0$) (see Tables 6-1). The color pattern of the *E. p. punctatolineatus* consists of a light brown, dorsal ground color with anterior, dorsal, dark crossbars, interrupted on the dorsal midline, which become reduced to dark spots posteriorly and then progressively change to longitudinal continuous or interrupted dorsal parallel lines at 1/2 to 2/3 of the total length. This typical pattern differs a little in some specimens where the anterior dorsal crossbars are replaced by square-shaped dark spots. Some adults are completely patternless. In a few adult specimens from Akdamar Island in Lake Van, the dorsal ground color is different, being dark gray or black (the former melanistic subspecies *kumerloevae* Eiselt, 1970); and these specimens don't display any visible dorsal pattern.

Distribution: Central-eastern Turkey, central and southern Armenia, southern Azerbaijan, Nakhichevan, and northern and western Iran in the northern Zagros and Azerbaijan mountains and throughout the Alborz Mountains up to northern Khorasan and the adjacent mountains of central Iran.

6-4-4- *Eirenis (Pediophis) punctatolineatus condoni* (Boulenger, 1920) new combination

Contia condoni Boulenger, 1920; J. Bombay Nat. Hist. Soc. 6 (4): 1024

Terra typica: Around Shiraz, Fars province, Iran. *Contia condoni* was described by Boulenger (1920) based on a couple of specimens, collected by V. Condon. The female specimens were presented originally to the Bombay Natural History Society Museum but the male was deposited in the British Museum. Because the specimens have been killed roughly, they are not in a satisfactory condition (Boulenger, 1920).

Diagnosis: *Eirenis punctatolineatus condoni* (Boulenger, 1920) can be distinguished from *Eirenis punctatolineatus punctatolineatus* (Boettger, 1892) by a combination of morphometric, meristic and color pattern characters. *Eirenis punctatolineatus condoni* has a higher mean value in the number of subcaudal scales than the *Eirenis punctatolineatus punctatolineatus*, 72 – 93 ($X = 83.46$) compared to 63 – 77 ($X = 72.09$)

in males and 72 – 88 ($X= 77.38$) compared to 61 – 68 ($X=64.67$) in females. Also, *E. p. condoni* has a higher ratio of tail length to total length than *E. p. punctatolineatus*, 26.65 – 30.70 ($X= 28.60 \pm 0.29$) compared to 21.86 – 26.40 ($X=24.66 \pm 0.40$) in males and 23.53 – 27.73 ($X= 25.46 \pm 0.43$) compared to 20.99 – 23.17 ($X=22 \pm 0.36$) in females. *E. p. condoni* has a lower ratio of head length to total length in males than *E. p. punctatolineatus*, 3.24 – 4.39 ($X= 3.81 \pm 0.11$) compared to 3.36 – 5.43 ($X= 4.13 \pm 0.17$). Also, the ratio of snout length to total length in females *E. p. condoni* is significantly lower than in females *E. p. punctatolineatus*, being 1.04 – 1.82 ($X= 1.3 \pm 0.09$) compared to 1.38 – 1.62 ($X= 1.49 \pm 0.03$). Contrary to the *E. p. punctatolineatus*, the hind body of *E. p. condoni* has no pattern.

Distribution: *Eirenis punctatolineatus condoni* is distributed in the southern Zagros mountains and the adjacent area including the western Zagros foothills, low elevated mountain sides adjacent to Persian Gulf, and eastwards to around Kerman and Bam cities in Kerman province.

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CHAPTER 7

Review of Taxonomy and Distribution of the *Eirenis medus* Species Complex (Chernov, 1940) (Serpentes: Colubridae) with Description of a New Species of the Genus *Eirenis* from Kerman Province, Southeastern Iran.

Modified from: Rajabizadeh, M., Schmidtler, J. F., Orlov, N. & G. Soleimani. (2012). Review of taxonomy and distribution of the *Eirenis medus* group (Chernov, 1940) (Ophidia: Colubridae) with description of a new species of the Genus *Eirenis* from Kerman Province, Southeastern Iran. Russian Journal of Herpetology, 19(4): 307-313.

Abstract

Specimens of four populations of the *Eirenis medus* species complex from Hamedan province in western Iran, Isfahan province in central Iran and Kerman province in southeastern Iran have been examined morphologically and compared with typical *Eirenis medus* in Turkmenistan and northeastern Iran. Based on morphological data, specimens of Kerman province differ from known populations of *Eirenis medus* in morphometric and meristic characters and should be regarded as a different taxon. As a result, *Eirenis kermanensis* is described based on the examination of a couple of specimens from the Sarduih region, Kerman Province, southeastern Iran. Compared with *Eirenis medus*, *Eirenis kermanensis* has a higher number of subcaudals and a higher ratio of tail length over total length. Also, in *Eirenis kermanensis*, the loreal scale is deeper than long, while in *Eirenis medus* the loreal is longer than deep. Further studies are necessary to determine the taxonomy of specimens Hamedan and Isfahan province.

Keywords: *Eirenis medus*, *Eirenis kermanensis*, Kerman province, Sarduih region, Iran.

7-1- Introduction

Eirenis medus was described originally as *Contia meda* from Kopet Dag in Turkmenistan by Chernov (1940). It is a member of the subgenus *Pediophis* and is related to the eastern Anatolian species *Eirenis thospitis* Schmidtler and Lanza, 1990 and *Eirenis hakkariensis* Schmidtler et Eiselt, 1991 (Nagy *et al.*, 2003). The species is known from Turkmenistan and northern Iran (Ananjeva *et al.*, 2004; Terent'ev and Chernov, 1949). Its distribution in Iran is however not clear because Latifi's description of the species is not correct. Except from findings of the species through the northern Iran mountains, Latifi (1991, 2000) recorded some doubtful localities of the species at the Iran-Turkey border and in Khuzestan Province in southwestern Iran (Figure 7-1). Also, a record of the species from southeastern Iran was noted by Szczerbak (2003). Since 2007, different populations of dwarf snakes, related to *Eirenis medus*, were collected throughout the Zagros mountains and adjacent areas, or were identified in the collections. Taxonomy of these specimens were not clear. In this study we tried to investigate the taxonomy of the *Eirenis medus* species complex populations in Iran using a morphological approach.

7-2- Materials and Methods

During field expeditions and collection examinations since 2007, eight specimens of the *Eirenis medus* species complex from three localities have been examined. A couple of specimens from the Sarduih region, Kerman Province, southeastern Iran were collected by G. Soleimani at the beginning of May 2008 (Figure 7-2 till 7-4). Also, three specimens were collected from Isfahan Province by M. Rajabizadeh in June 2011 (Figure 7-5). The collected specimens were preserved in 75% alcohol, with following reference numbers: Kerman specimens: ICSTZM 7H1062 and 1064, Isfahan specimens ICSTZM 1118, 1123 and 1130.

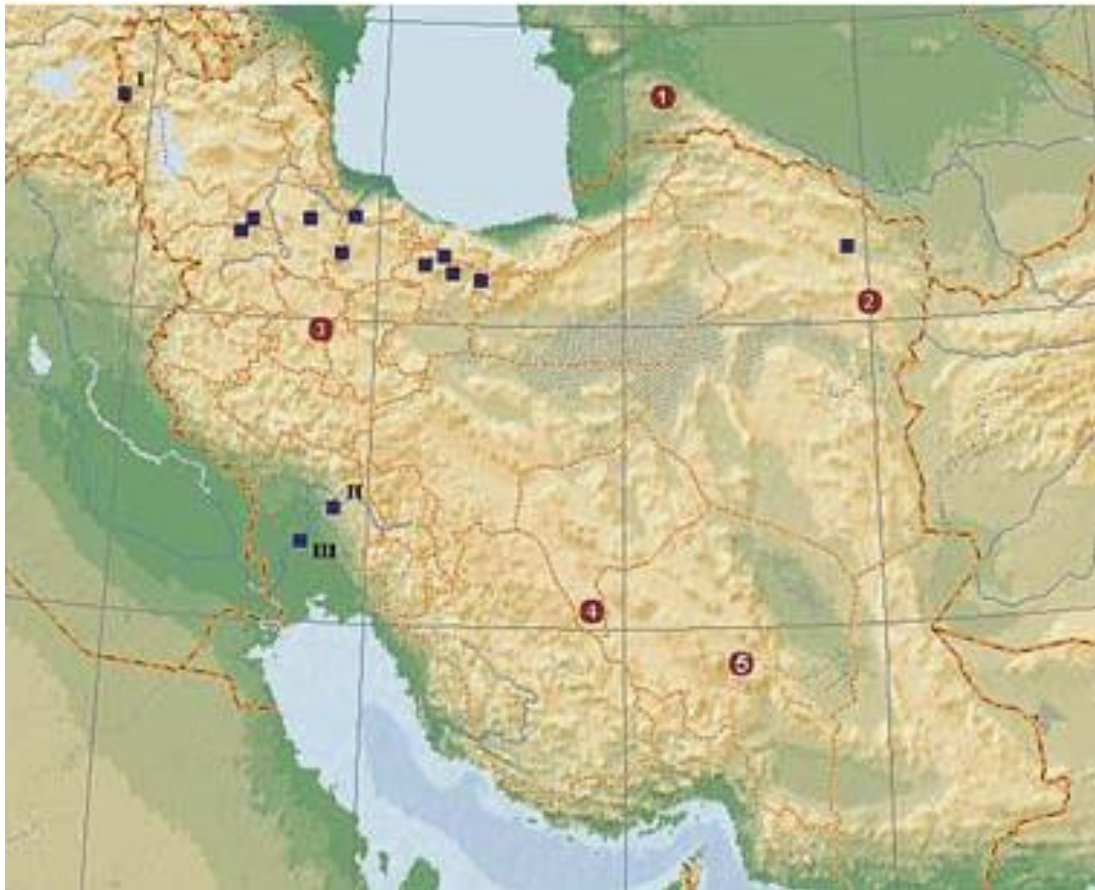


Figure 7-1. Distribution map of examined specimens of *Eirenis medus* (Nrs. 1 and 2 in Turkmenistan and northeastern Iran) and the *Eirenis medus* species complex (Nrs. 3, 4, and 5 through the Zagros mountains and adjacent areas), as well as Latifi's (1991 and 2000) records of *Eirenis medus* in Iran. Localities of examined specimens are in circles: 1, Kopet Daggh population in Turkmenistan; 2, Khorasan population in northeastern Iran; 3, Hamedan population, Hamedan province, Iran; 4, Isfahan population, Isfahan province, Iran; 5, newly found population in Kerman Province, Iran. Localities of Latifi's (1994 and 2000) records are in quadrates, three of them are very doubtful and strange (see text): I, Ghotur; II, Masjed Soleiman; III, Ahwaz.



Figure 7-2- Dorsal (a) and ventral (b) view of the body in the holotype of *Eirenis kermanensis*.



Figure 7-3 Dorsal (a) and lateral (b) side of the head in the holotype of *Eirenis kermanensis*.



Figure 7-4- Lateral side of head (a) and view of the dorsal body (b) in the paratype of *Eirenis kermanensis*.



Figure 7-5- Dorsal body of a *Eirenis medus* species complex specimen from Kopet Dag, Turkmenistan (a), Isfahan province (b), and Hamedan province (c)

Also, following museum specimens of *Eirenis medus* species complex were examined: three specimens from Soltan Bolagh, around Hamedan city, Hamedan province, western Iran (MHNG 2627 2 – 4); eight specimens from Kopet Dag mountain in Turkmenistan and Khorasan province in northeastern Iran (ZISP 9270, 8458, 8440, 8461, 9271, ZSM 1175-1176/2006) (Figure 5).

Specimens were examined for four metric morphological characters including: four metric characters (SVL, TL, HL, HW), two meristic characters (Ven, Scd), nine binary and categorical characters (Pre, Sq, Pre.Oc, Post.Oc, Lor, Tem, Sup, Lor.s) (see Table 2-1 for definitions). For the comparison of metric characters, ratios were used: TL/ToL, HL/ToL, HL/HW, SL/SW (see Table 2-1 for definitions).

To reveal character patterns of the different populations, descriptive statistical parameters including minimum, maximum, mean and standard error (S.E.) were calculated (Table 7-1). To explore the geographic difference within the *Eirenis medus* species complex, between-groups PCA based on the correlation matrix, were run on metric and meristic characters of all the examined specimens. Principal components were checked until the level of 95% of the total variation.

7-3- Results

7-3-1- Distribution and morphology of the *Eirenis medus* species complex

Based on this first description and on the examined specimens, the distribution of *Eirenis medus* seems to be restricted to Turkmenistan and adjacent areas in northeastern Iran.

7-3-2- Morphological comparison

The specimens of Kopet Dag and northeastern Iran show similar morphological features with the type specimens (see Chernov, 1940), whereas those from Hamedan, Isfahan and Kerman provinces, being referred to as belonging to the *Eirenis medus* species complex, differ from the Turkmenistan's populations of *Eirenis medus* (Table 7-1) (Figure 7-6).

Table 7-1- Descriptive parameters of morphological characters including Minimum, Maximum, Mean, and Standard Error across populations of *Eirenis medus* species complex. The ratio characters were multiplied by 100.

Character	Turkmenistan		NE Iran		Hamedan	Isfahan		Kerman	
	3	3	1	1	3	1	2	1	1
TL/TOL	21.28 ± 0.44 20.73 – 22.57	16.99 ± 0.40 16.37 – 18.09	20.74	18.09	19.59 ± 0.57 18.45 – 20.12	23.47	20.90 ± 0.28 20.62 – 21.18	25	22.07
HL/TOL	4.06 ± 0.20 3.67 – 4.35	3.70 ± 0.04 3.61 – 3.81	—	3.61	4.19 ± 0.8 4.04 – 4.28	3.66	3.78 ± 0.11 3.67 – 3.88	3.86	3.44
HW/HL	67.59 ± 2.71 62.85 – 72.25	62.91 ± 5.16 47.5 – 69.49			65.3 ± 1.92 62.60 – 69.02	63.59	59.60 ± 3.62 55.98 – 63.21	60.90	61.32
Pre	1 1 – 2	1 0 – 2	2	2	1.33 0 – 2	1	2	1	0
Ven	155 ± 1.41 151 – 157	171 ± 1.87 166 – 175	155	166	150.66 ± 0.66 150 – 152	154	164 ± 1.0 163 – 165	154	165
Scd	52.75 ± 0.48 52 – 54	45.5 ± 0.96 44 – 48	52	46	47.33 ± 0.33 47 – 48	60	56 ± 0.0	63	59
Sq (m)	15	5	15	15	15	15	15	15	15
Pre.Oc. (R/L)	1 1	1 1	1 1	1 1	1 1	1 1	1 1	1 1	1 1
Post.Oc. (R/L)	2 2	2 2	1 2	1 2	2 2	2 2	2 2	2 2	2 2
Lor (R/L)	1 1 1 + 2/1+2	1 1 1 + 2/1+2	1 1 1 + 2/1+2	1 1 1 + 2/1+2	1 1 1 + 2/1+2	1 1 1 + 2/1+2	1 1 1 + 2/1+2	1 1 1 + 2/1+2	1 1 1 + 2/1+2
Tem 1+2 (R/L)									
Sup. (R/L)	7 7	7 7	7 7	7 7	7 7	7 7	7 7	6 6	6 6
Inf. (R/L)	8 8	8 8	8 8	8 8	8 8	8 8	8 8	8 8	7 7
Lor.s	No	No	No	No	No	No	No	Yes	Yes

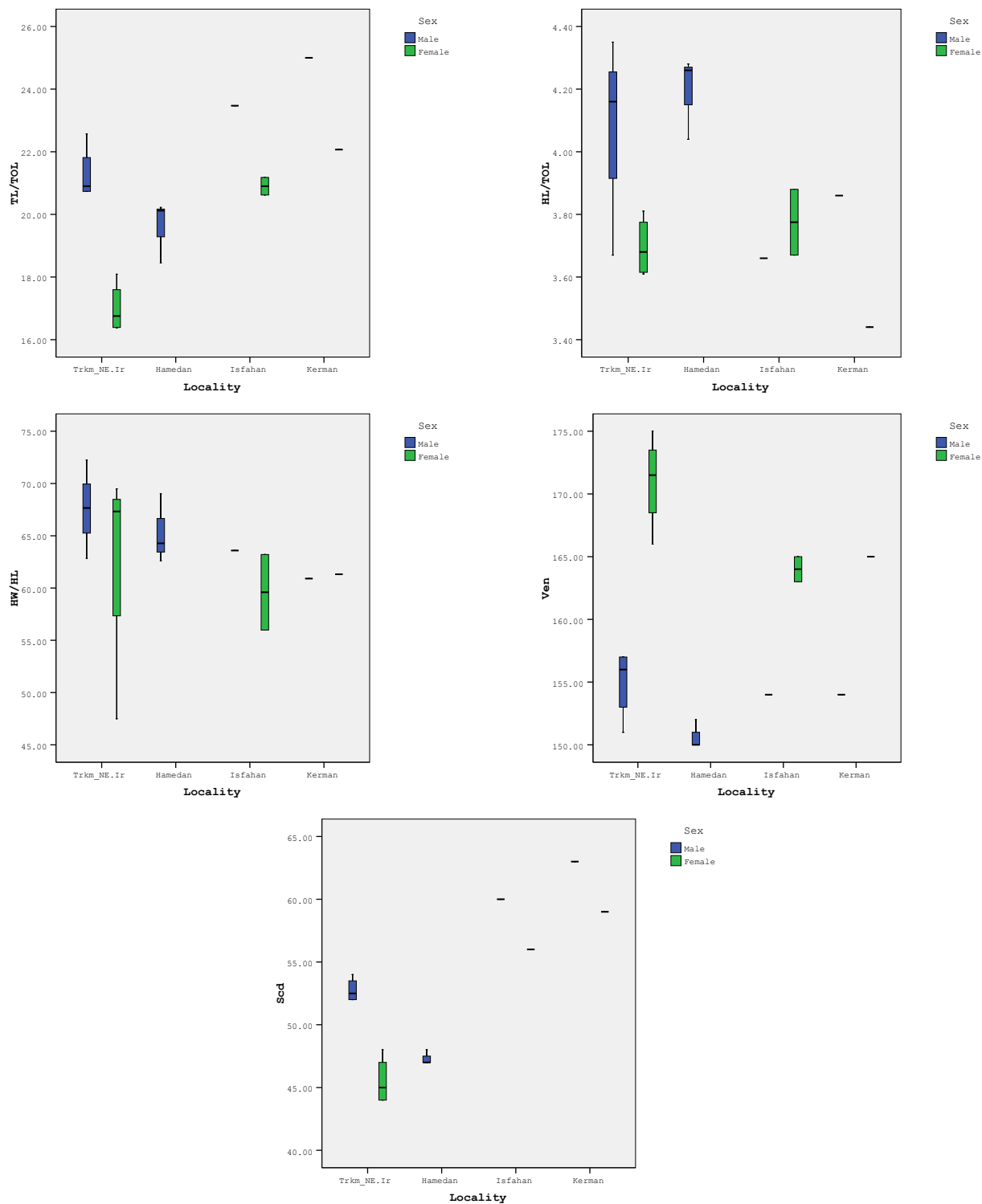


Figure 7-6- Box plots showing the minimum, first quartile, median, third quartile, and maximum for the morphological characters across populations of the *Eirenis medus* species complex. The ratio characters were multiplied by 100. The scatter plot resulting from the between-groups PCA on specimens of the *Eirenis medus* species

complex (Figure 7-7) reveals that Kerman specimens diverge from all other populations by the ratio of tail length over total length and number of subcaudal scales. The specimens from Hamedan and Isfahan are close to the *Eirenis medus* specimens of Turkmenistan and northeastern Iran, but geographically they are far from each other. The conclusion is that the various morphological differences in morphometric and meristic characters of the Kerman *Eirenis* specimens suggest the taxonomic separation of the Kerman specimens.

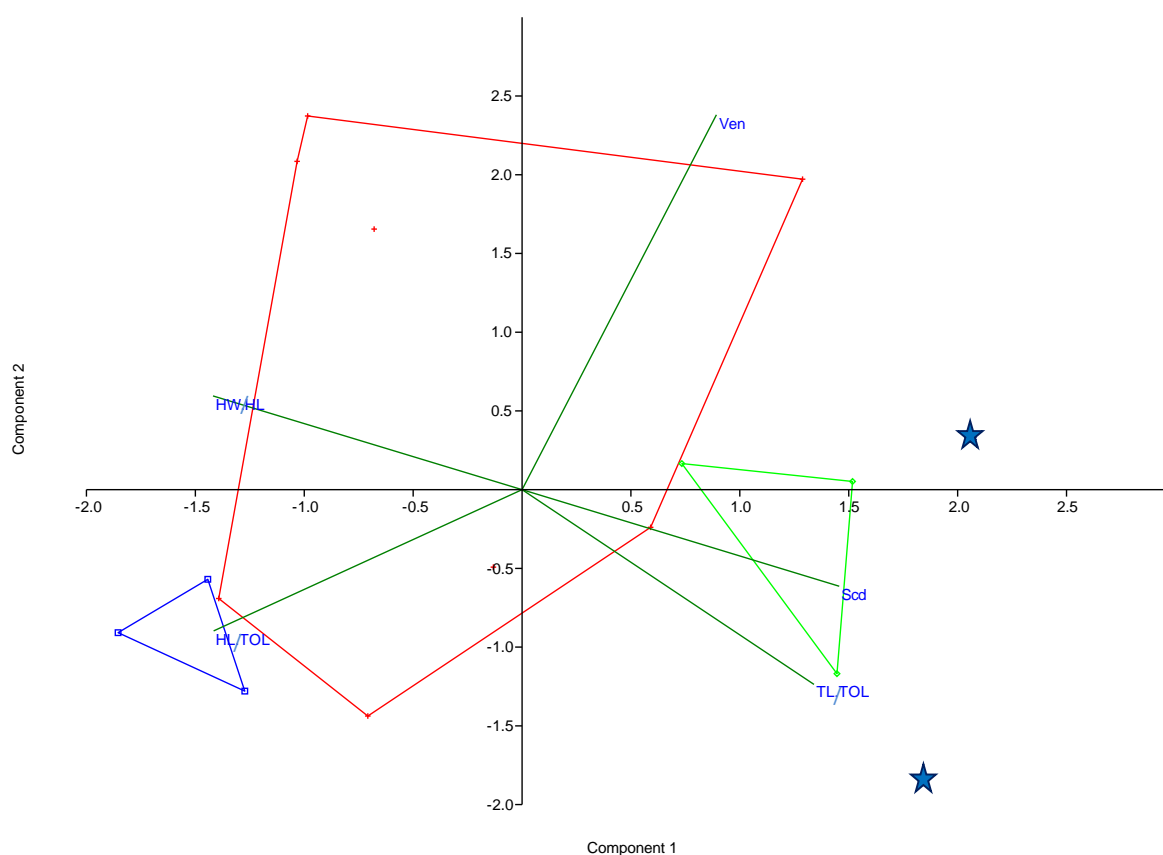


Figure 7-7. Scatter plot resulting from the between groups PCA showing the divergence of the Turkmenistan-northeastern Iran (red), Hamedan (blue), Isfahan (green) and Kerman (blue star) populations of the *Eirenis medus* species complex. All variables and their contribution (factor loading) in population divergence are superimposed on the plots. The first component covers 78.71% and the second component cover, 19.75% of the total variation.

7-3-3- Taxonomic account

A question to be asked is that if this amount of difference separates the Kerman specimens from *Eirenis medus* at the subspecies or whether the species level. A review of formerly described species in the genus *Eirenis* shows that various closely related species in this genus are separated from each other by few morphological differences (*Eirenis rechingeri* Eiselt 1971, *Eirenis rothii* Jan 1863, *Eirenis eiselti* Schmidtler and Schmidtler 1978, *Eirenis barani* Schmidtler 1988, *Eirenis hakkariensis* Schmidtler and Eiselt 1991, *Eirenis thospitis* Schmidtler and Lanza 1990, and *Eirenis levantinus* Schmidtler 1993). Due to this background, the authors propose that the Kerman specimens, differing from the closely related *Eirenis medus* by a combination of morphometric and meristic characters (see diagnosis, 7-4-3), and occurring distantly and isolated from the known distribution of *Eirenis medus*, require a separate species rank. On the other hand, the taxonomy of the specimens of Hamedan and Isfahan, displayed above, is not clear. Additional studies are necessary to determine the taxonomy of these populations.

7-4- *Eirenis (Pediophis) kermanensis* (Rajabizadeh et al., 2012)

7-4-1- Holotype. Adult female, ICSTZM7H1064. Iran, Kerman province, Sarduih region, Babe Karafs village, (29°16 N 57°15 E), 2800 m; coll. Gh. Soleimani, May 2008 (Figures 7-2 and 7-3).

7-4-2- Paratype. Adult male, ICSTZM7H1062. Same data as holotype (Figure 7-4).

7-4-3- Diagnosis. *Eirenis kermanensis* (Rajabizadeh et al., 2012) differs from *Eirenis medus* by a combination of meristic and morphometric characters. Meristically, the Kerman specimens have a higher number of subcaudal scales (63 in males, compared to 52 – 54 (52.75 ± 0.48) in males of *Eirenis medus* and 59 in females, compares to 44 – 48 (45.5 ± 0.96) in females of *Eirenis medus*). The number of labial scales in *Eirenis kermanensis* shows a tendency for reduction (six supralabials, instead of seven in *Eirenis medus* (Table 7-1). In morphometric characters, the percent ratio of tail length on total length (TL/TOL) in the new *Eirenis* is higher than that in *Eirenis medus* (25 in males, vs. 20.73 – 22.57 (21.28 ± 0.44) in *Eirenis medus*; 22.07 in females vs. 16.37 – 18.09 (16.99 ± 0.40) in *Eirenis medus*. Additionally, in *Eirenis medus*, the loreal scale is longer than deep, whereas in the new *Eirenis* species, the loreal scale is deeper than long (Figure 7-3). Concerning the color pattern, in *Eirenis kermanensis* the dorsal crossbars at mid-body are wider than in *Eirenis medus* in type locality (one scale wide in *Eirenis kermanensis* compared to half a scale wide in *Eirenis medus* (Figure 7-5).

7-4-4- Description of holotype

Pholidosis. Head covered dorsally with large scales: rostral only slightly visible from above, internasal slightly shorter in length than prefrontal, frontal about the same length as the inter-parietal suture, posterior edge of parietals somewhat in parallel to posterior border of supralabials; parietals longer than frontal. On the side of the head, the nostril is in the middle of an elongate semifused nasal scale; loreals are very small and deeper than long (downward, long, triangle shape in the right side and somewhat long, rhomboid shape in the left side); six supralabials, third and fourth bordering eye, sixth is largest; a single preocular, two postoculars, the lower one slightly bigger; one anterior and two posterior temporals; seven infralabials, fourth has a trapezoid shape and is the biggest, anterior four in contact with first pair of inframaxillaries; posterior inframaxillaries shorter than anterior pair, contacting each other. Head rounded, body and tail slender, 165 ventral plates are followed by 56 pairs of subcaudal scales, anal plate divided, no preventral. There are six scales between the first ventral and the posterior pair of inframaxillaries. Dorsal scales are smooth and dorsal scale rows at one head length after head, midbody and one head length before anus are in 15 longitudinal rows.

Morphometric characters. Snout vent length is 240 mm, tail length 68 mm, head length 10.6 mm and head width 6.5 mm.

Color pattern. Dorsal head ground color is light brown with irregular, black blotches on frontal, supraocular, parietal and temporal. On the side of the head, scales are light brown and irregular, with black blotches scattered mainly at the margin of scales around the eye, upper supralabial scales and suture of supralabials. Dorsal body and tail ground color is light brown with parallel, black, dorsal bands the width of which is slightly larger than the length of one dorsal scale. Dorsal bands run regularly from nape to anterior one third of tail; on mid-dorsum, dorsal bands are interrupted and sometimes appear as a zigzag arrangement. Quadrate-shaped black blotches are repeated in the dorsolateral zone in parallel to the dorsal cross bars. Dorsal crossbars fade on posterior two thirds of tail to black spots. Ventral side of body and head is yellowish; especially at midbody, black spots are visible on both sides of the ventral plates (Figs. 7-2 and 7-3).

7-4-5- Variation. The morphological characters of the only paratype, an adult male are presented in Table 7-1. The metric characters of this specimen are: snout vent length 258 mm, tail length 86 mm, head length 13.3 mm and head width 8.1 mm. Habitus, color and pattern of the specimen are shown in Figure 7-4.

7-4-6- Etymology. *Eirenis kermanensis* is named after Kerman Province, the largest province of Iran in its southeastern part, with very different ecosystems from high mountains in the west to a hot desert in the east and from high elevated plains in the north to low coastal plains in the south. The herpetofauna of this review has been studied poorly up to now. With this naming, the authors hope that more herpetologists will pay attention to the herpetofauna, and especially the snake fauna, of this province.

7-4-7- Habitat. The habitat of *Eirenis kermanensis* is located at the mountainous sides of a high mountain valley at the elevation of 2800 m. The bottom of the valley is about 2600 m and is surrounded by mountains up to 3500 m in the north (Hezar mountains) and about 3000 m in the south (Bahraseman mountain). The habitat is composed of igneous stones and sandy soil. The vegetation of the locality is characterized by a high density of *Artemisia* sp. and scattered trees of *Amygdalus* sp. and *Pistacia terebinthus*. The climate of the area is cold mountainous (Figure 7-8).



Figure 7-8. Type locality of *Eirenis kermanensis* species near the Babe Karafs village, Sarduih region, Kerman province, southeastern Iran.

7-4-8- Comparison and relationships. About 18 generally accepted species have been identified in the genus *Eirenis* (www.reptiledatabase.org). A brief comparison of *Eirenis kermanensis* with other known species of *Eirenis* is as follows:

Eirenis kermanensis differs from *Eirenis (Pseudocyclophis) persicus* by having 1+2 temporal scales, while *Eirenis (Pseudocyclophis) persicus* specimens have 1+1 temporal scales. *Eirenis kermanensis* has 15 dorsal scales and so it is simply distinguished from the following *Eirenis* species in having 17 dorsal scales: *Eirenis lineomaculatus*, *Eirenis punctatolineatus*, *Eirenis hakkariensis*, *Eirenis africanus*, and *Eirenis modestus*.

Compared to *Eirenis* species with 15 dorsal scales (all species belonging to the subgenus *Pediophis*), *Eirenis kermanensis* is distinguished from some of them simply in color pattern. These species include *Eirenis rechingeri*, characterized by a dorsolateral dark strip, and *Eirenis collaris*, characterized by a dark transverse strip on the collar region. *Eirenis rothii*, *Eirenis eiselti*, *Eirenis levantinus*, and *Eirenis barani* are usually characterized by an unicolor body and dark head patterns, which can simply be distinguished from the *Eirenis kermanensis* color pattern.

Eirenis kermanensis is distinguished from *Eirenis coronelloides* in the latter forming a lower number of subcaudal scales, lower than 50 scales, whereas the number of subcaudals in the new species is higher than 50 subcaudals.

The East Anatolian *Eirenis thospitis* differs from *Eirenis kermanensis* in the lack of a dorsal pattern in adults, as well as in its high number of ventral scales (*Eirenis thospitis* has 169 – 190 ventral scales, compared to 154 – 165 in *Eirenis kermanensis*).

Eirenis coronella, which has been reported from the southwestern lowlands of Iran, is distinguished from *Eirenis kermanensis* by its dorsal color pattern having a dark, collar-shaped stripe on the neck, which is in contact with a dark dorsal head dark blotch. This distinct pattern is not seen in the new species.

Morphological differences between *Eirenis kermanensis* and *Eirenis medus* have been discussed in detail above.

The authors hope that upcoming molecular data of *Eirenis kermanensis* will further enlighten more the systematic position of this new species.

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CHAPTER 8

Taxonomy of *Eirenis (Pseudocyclophis) persicus* (Anderson, 1872) (Serpentes: Colubridae)

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Abstract

The dwarf snake *Eirenis (Pseudocyclophis) persicus* (Anderson, 1872) has a wide distribution range in south western Asia. As is inferred from the list of claimed synonyms, *Eirenis persicus* species complex is a species complex that was not subjected to a sufficiently thorough comparative study yet. In this study, 23 male and 17 female specimens of the *Eirenis persicus* species complex were studied using both traditional biometry and geometric morphometrics on landmark data of dorsal head scales. Two mitochondrial genes, 16S rRNA and cytochrome *b*, and one nuclear gene, *c-mos*, were sequenced and then a concatenated data set of them was analysed to generate a Bayesian inference of phylogeny. Analyses revealed that the populations of *Eirenis persicus* in the western mountains of Iran and Turkey are significantly different from the populations in eastern Iran, Turkmenistan and Pakistan. This significant divergence is confirmed by geometric morphometric and molecular analyses. Both molecular and morphological analyses showed, specimens of the *Eirenis persicus* species complex, with *nigrofasciatus* color morph, are closely related to the eastern populations of the *Eirenis persicus* species complex. Within-groups PCA revealed that *Eirenis persicus* populations of Iran and Turkey are morphologically different from each other at a subspecific level. This result was confirmed by molecular data as well.

Key Words: *Eirenis persicus*, *Pseudocyclophis*, *nigrofasciatus*, *walteri*, taxonomy, geometric morphometrics, 16S rRNA, cytochrome *b*, *c-mos*.

8-1- Introduction

The dwarf snake *Eirenis (Pseudocyclophis) persicus* (Anderson, 1872) has a wide distribution range, including the southern and southeastern Turkey, southern Armenia, eastern Iraq, Iran, southern Turkmenistan, and perhaps also parts of Afghanistan, Pakistan and northwestern India (Ananjeva *et al.*, 2004; Szczerbak, 2003; Khan, 2002; Latifi, 2000; Sindaco *et al.*, 2000). This secretive, small-sized (25-50 cm) and slender snake can be morphologically characterized by having 1 pre- and 1 postocular scale on the right and the left side of the head, usually no loreal scale, usually 7 supralabials, 1+1 temporals, 15 rows of dorsals at mid-body, 183 – 212 (♂), 202 – 231(♀) ventrals and 52 – 96 (♂), 45 – 82 (♀) subcaudals (Schmidtler and Schmidtler, 1978). Three different color morphs have been reported for this species, which are referred to as *persicus*, *walteri* and *nigrofasciatus* morphs. The *persicus* morph refers to specimens which have dark blotches on the dorsal head and neck and have a uniform body. The dark markings on the head result from a dark inter-ocular blotch and a dark parietal blotch. The head blotches may fuse to each other and to a collar blotch to form a continuous dark marking on the head, or they may be separated. The *walteri* morph has pale dark or blackish blotches on the head like *persicus*, but also has narrow dorsal crossbars on the anterior dorsum, which disappear towards the posterior body. The *nigrofasciatus* morph also has dark blotches on the head and neck, followed by distinct dorsal black bands on the body and tail (Figure 8-2).

Although *Eirenis persicus* has the widest distribution range in the genus, its taxonomy was so far not subjected to a critical taxonomic revision. In the present taxonomy, two subspecies are being recognized. All *Eirenis persicus* specimens of the *persicus* and *walteri* morph are referred to as *Eirenis persicus persicus* (Anderson, 1872). This classification is adopted from Dotsenko's studies in Turkmenistan (Dotsenko, 1986 a,b in Szczerbak, 2003) who showed that specimens with a *walteri* pattern are mainly females while specimens with a *persicus* pattern are mainly males. The other subspecies is *Eirenis persicus nigrofasciatus* (Nikolsky, 1907), which although being morphologically completely distinct, its validity has been doubted by several authors (see Haas and Werner, 1969) due to its overlapping distribution with *Eirenis persicus persicus*.

Eirenis persicus was originally described as *Cyclophis persicus* Anderson, 1872, from the southwestern Iranian Plateau (around Bushehr). At the generic level, the species was later placed in the genus *Pseudocyclophis* Boettger, 1888, then *Contia* Boulenger, 1894 and then *Eirenis* Jan, 1863. Frynta *et al.* (1997) suggested to re-evaluate the genus *Pseudocyclophis* 1888 again, but molecular studies by Nagy *et al.* (2003) showed that *Eirenis persicus* is of the basal lineages within the genus *Eirenis*. *Pseudocyclophis* is accepted as a monotypic subgenus in the genus *Eirenis* with *Eirenis (Pseudocyclophis) persicus* (Nagy *et al.*, 2003). After the description of *Eirenis persicus* by Anderson in 1872, *Contia angusticeps* from north western Pakistan (about 2000 km from *Cyclophis persicus*) was described by Boulenger, 1894. *Contia angusticeps* was regarded as a synonym of *Eirenis persicus* by Smith (1935). *Contia mcmahoni* was described based on specimens from eastern central Pakistan by Wall (1911). Because of the morphological similarity with *Eirenis persicus* and because of the type series being lost and no other specimens with similar morphological characters having been collected afterwards, this species was also put in synonymy with *Eirenis persicus*. *Pseudocyclophis walteri* Boettger, 1888 was described from around the border of Iran and Turkmenistan (around Sarakhs city) based on differences in color pattern with *Eirenis persicus*. Then Wall (1923) described *Contia zebrina* based on similar arguments. Both these species were also regarded a synonym of *Eirenis persicus*, following Dotsenko (1986 a,b). All available localities of *Eirenis persicus*, examined in this study, present in the collections or reported in the literature, are shown in Figure 8-2.

As is inferred from the list of claimed synonyms, *Eirenis persicus* species complex is potentially a species complex that was not yet subjected to a sufficiently thorough comparative study, and it seems that different taxa from a wide geographic distance were lumped together into *Eirenis persicus persicus*. In this paper we provide a comprehensive review on the taxonomy of this *Eirenis persicus* species complex. We especially focus on the taxonomic status of the different morphs that can be recognized within this species complex.

8-2- Materials and methods

Based on extensive field expeditions throughout Iran, and examination of the museum specimens, a total of 23 male and 17 female Specimens were examined. Iranian specimens were obtained from the following collections: ICSTZM, RUZM, CBSU, DHZC and DEZC. Specimens from Turkey, Turkmenistan and Pakistan originate from the BMNH, CAS, ZSM and ZMMU. For complete names of the abbreviations, see chapter 2, materials and methods). Color and pattern of the examined specimens, as well as localities are presented in figures 8-1 and 8-2.

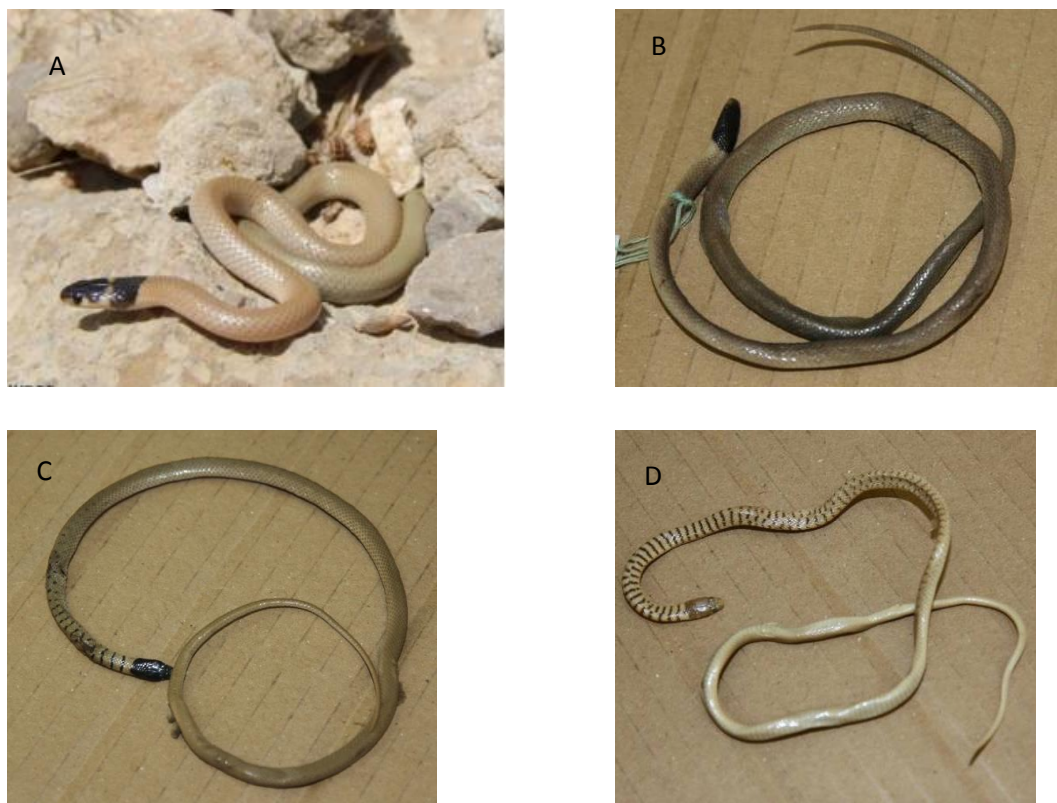


Figure 8-1. Different morphs of *Eirenis persicus* species complex: A: *persicus* morph from southwestern Iran (Fars province), B: the *persicus* morph from Turkmenistan, C and D: *walteri* morph from Turkmenistan and southwestern Iran (Kerman province), E: *Eirenis persicus nigrofasciatus* (from southern Iran), F: New morph from the central Zagros mountains (Sisakht), Iran (single specimen found). Continued on the next page.



Figure 8-1 continued. Caption on the previous page.

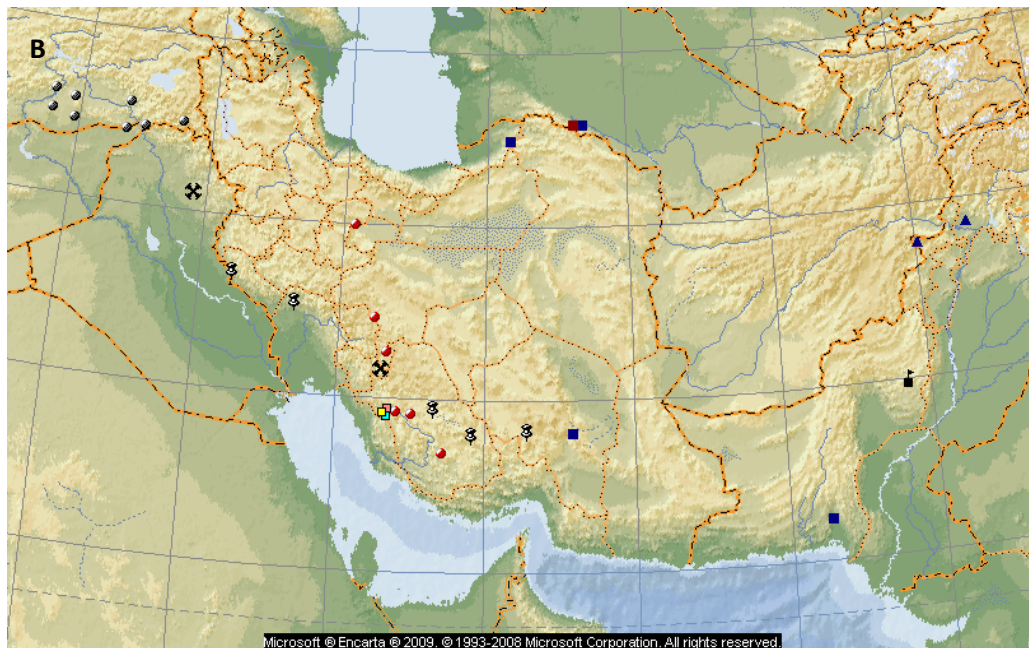
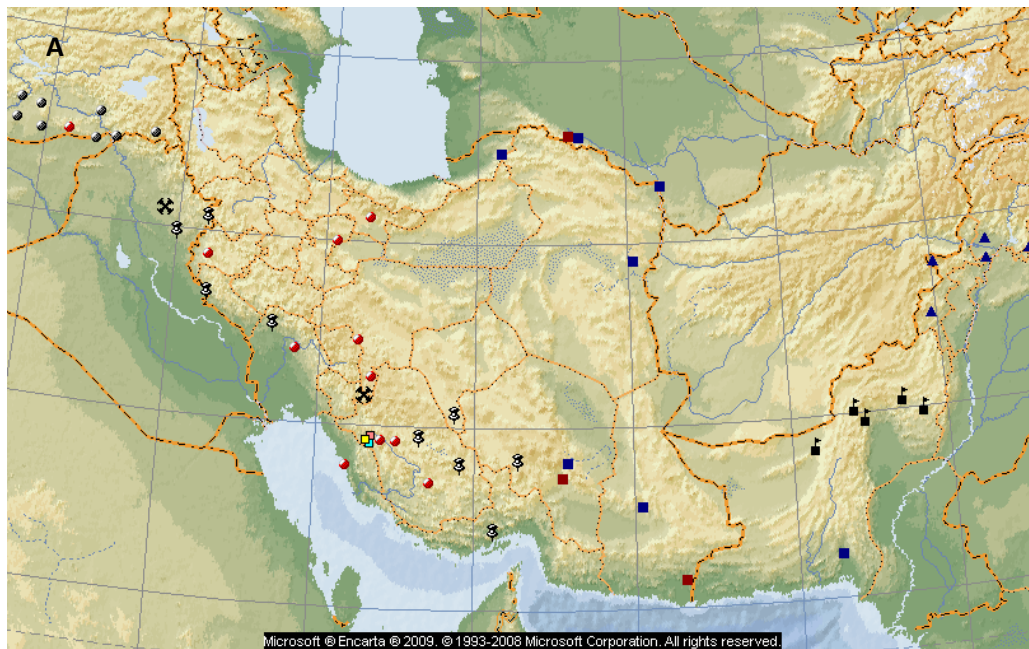


Figure 8-2. A: All available distribution records of the *Eirenis persicus* group. Circle: *Eirenis persicus* from western Iran (red) and Turkey (gray); square: *Eirenis persicus* from eastern Iran, Turkmenistan and Pakistan, with both *walteri* morph (blue) and *persicus* morph (red); cross: *Eirenis persicus* with the new pattern; pin: *Eirenis persicus nigofasciatus*; triangle: *Eirenis anguisticeps* (sensu Boulenger, 1894); flag: *Eirenis mcmahoni* (sensu wall, 1911). B: map showing localities of the specimens examined for this study.

23 male and 17 female specimens were examined for 15 morphological characters: six metric characters (SVL, TL, HL, HW), two meristic characters (Ven, Scd), seven binary and categorical characters (Sup, Inf, pre, Lor, bet.l.inf, bet.po.chin, hind.par, Sq.F, Sq.H) (see Table 2-1 for definitions). Because in the eastern OTU, most of the specimens with the *walteri* pattern are female (only one exception in this study) and all specimens of the *persicus* morph are male, both sexes were pooled for the multivariate analysis. For the descriptive analysis and graphic presentation of the metric data, ratios were used: TL/TOL x 100, HL/TOL x 100, HW/TOL x 100 (see Table 2-1 for definitions).

As *Eirenis persicus* is distributed across a wide range in southwestern Asia, and because the species has different color patterns, the specimens were grouped into OTUs based on color morph and geographic distance between the populations. (See subtitle 8-3-1, for the list of OTUs).

To investigate whether these OTUs are characterized also by morphological differences, using metric and meristic characters, all OTUs were compared by between-group principal component analysis (BG-PCA) based on the correlation matrix. Morphological characters that contribute to a separation of the OTUs in the PCA could then be identified. Principal components were checked up the level that they cumulatively explained at least 95% of the total variation. To test whether the OTUs were significantly different, a MANOVA was performed. For significantly different OTUs, discriminating characters between the OTUs were identified using an analysis of covariance (ANCOVA) for metric characters (total length as covariate) and analysis of variance (ANOVA) for meristic characters. For that, metric characters were tested as ratios, whereas for the non-parametric characters a Kruskal-Wallis test was run on the individual variables, combined with a multivariate npMANOVA on all the variables. OTUs with low number of samples were excluded from the statistical analysis

To explore occurrence of different taxa within the OTUs, specimens of the eastern and western OTUs were then compared using a within-group PCA based on the correlation matrix. This analysis was only done on the eastern and western OTUs, because these OTUs include specimens from a wide distribution range, involving different mountain systems. This analysis was not run on the *nigrofasciatus* OTU, as it is limited to the southern Zagros and adjacent areas in the western Zagros foothills. Individuals that after visual inspection of PCA plots were

showing a deviating position compared to the specimens of the same OTU were then checked again for the occurrence of different taxa. An overview of the phenotypic variation that characterize the different groups (including OTUs and possible groupings within OTUs) is given based on descriptive statistical parameters, including minimum, maximum, mean and standard error (S.E.) (Table 8-4; Figure 8-8).

A geometric morphometric analysis was performed using high quality pictures from the dorsal head of 46 specimens, on which 30 landmarks were put (Figure 8-3) and coordinates of the landmarks were saved using tpsDig2 package (Rohlf, 2010). Except for the border of the parietal scales, the landmarks were at the intersection of the sutures of three or more scales. (Figure 8-3). The landmark data was analyzed using a between-group PCA based on the variance-covariance matrix. Principal components were checked up to the level that they cumulatively explained at least 95% of the total variation, and the significance of the differences between the OTUs was checked using a npMANOVA test. Shape variation within and across OTUs was visualized by partial warp analysis and computing deformation grids in tpsRelw (Rohlf, 2003). The scales that contributed to the shape variation between OTUs will identify using visual inspection.

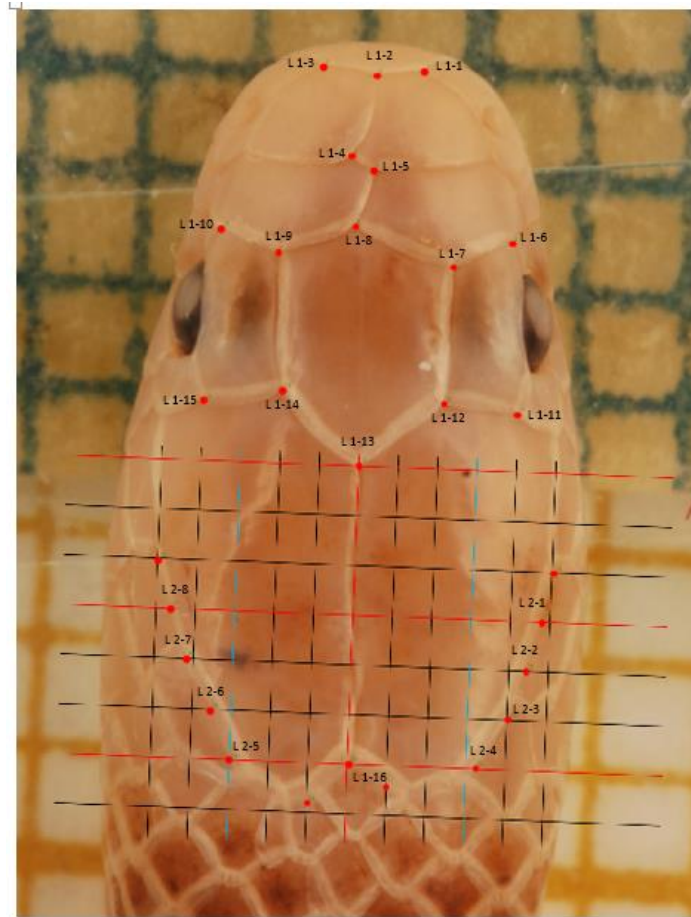


Figure 8-3. Landmarks that were used on the intersection of dorsal head scales in *Eirenis persicus* species complex specimens. The grid was used to put semi-landmarks on parietal scales. Definition of the type 1 landmarks. L1-1: intersection point of sutures between the right nasal, right internasal and rostral scale, L1-2: intersection point of sutures between the left and right internasal and rostral scale, L1-3: intersection point of sutures between the left nasal, left internasal and rostral scale, L1-4: intersection point of sutures between the left and right internasals and prefrontal, L1-5: intersection point of sutures between the left and right prefrontals and internasal, L1-6: intersection point of sutures between the right supraocular, right prefrontal and right preocular, L1-7: intersection point of sutures between the right supraocular, right prefrontal and frontal, L1-8: intersection point of sutures between the left and right prefrontals and frontal, L1-9: intersection point of sutures between the left supraocular, left prefrontal and frontal, L1-10: intersection point of sutures between the left supraocular, left prefrontal and left preocular, L1-11: intersection point of sutures between the right supraocular, right parietal and right postocular, L1-12: intersection point of sutures between the right supraocular, right parietal and frontal, L1-13: intersection point of sutures between the left and right parietals and frontal, L1-14: intersection point of sutures between the left supraocular, left parietal and frontal, L1-15: : intersection point of sutures between the left supraocular, left parietal and left postocular, L1-16: : intersection point of sutures between the left and right parietals and the closest hind parietal scale. Definition of the Type 3 landmarks: 8 landmarks were put on the outer edge of the parietal scales, along six horizontal lines, dividing the interparietal suture, named as 2-1 to 1-8.

For the molecular phylogenetic analyses, the majority of the sequences were from the study by Nagy *et al.* (2003). Here we add four new specimens of the *Eirenis persicus* group, including one specimen with a *walteri* pattern coming from southeastern Iran, one specimen with a *nigrofasciatus* pattern and two specimens with a *persicus* pattern, all coming from southwestern Iran. A concatenated data set of cytochrome *b*, 16S rRNA and c-mos sequences, including 33 samples and 2200 bp, was used for a Bayesian inference of the phylogeny, conducted in MrBayes v3.1.2 (Ronquist and Huelsenbeck 2003). In the phylogenetic analysis, genus *Hierophis* is considered as the most recent ancestor of the genus *Eirenis* (following Nagy *et al.*, 2003). Pairwise genetic divergences of the cytochrome *b* gene were used as supplementary quantitative data for assessment of the level of species delimitation (following Nagy *et al.*, 2012; Johns and Avise, 1998).

8-3- Results

8-3-1 Construction of Operational Taxonomic Units (OTUs)

The *Eirenis persicus* specimens were grouped into three OTUs based on color morph and geographic distance between the populations, including (1) a western OTU, comprising specimens of western Iran and Turkey, having *persicus* morph; (2) an eastern OTU comprising specimens of eastern Iran, Turkmenistan and Pakistan, having *persicus* and *walteri* morph; (3) a *nigrofasciatus* OTU comprising specimens of *Eirenis persicus nigrofasciatus* morph (from now on is referred to as *nigrofasciatus* OUT); (4) two specimens with a new color pattern were put into a separate OTU, named 'novum' (Figure 8-4). These specimens agree in overall morphology with the description of *Eirenis persicus*, but the color pattern of these specimens has not been reported for *Eirenis persicus* yet. Eastern and western OTUs are separated geographically by central Iran, arid plains which are not a suitable habitat for *Eirenis persicus*.

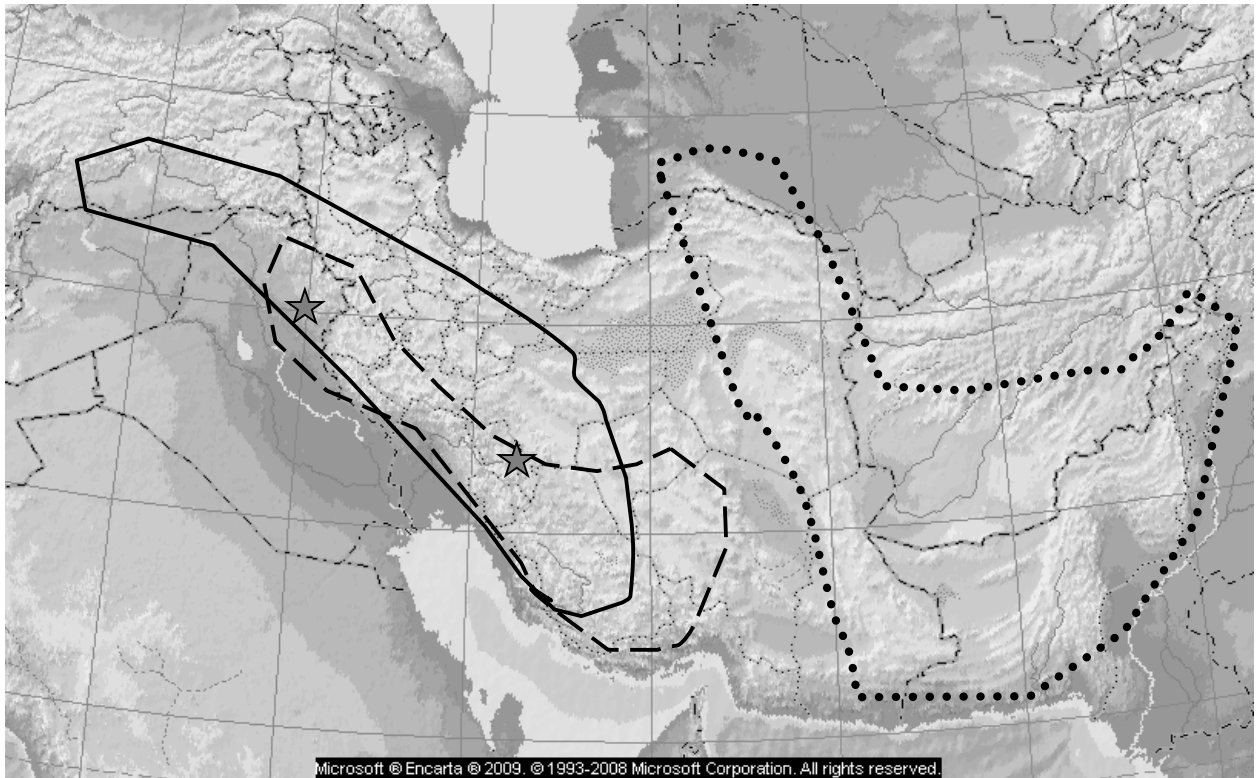


Figure 8-4. Geographic position of the different OTUs: eastern OTU (dotted line), western OTU (dashed line), *nigrofasciatus* OTU (solid line), novum OTU: star.

8-3-2- Multivariate and univariate comparison of OTUs

The BG-PCA scatter plot on parametric data of all four OTUs of *E. persicus* (Figure 8-5) reveals that the eastern OTU diverges from the western OTU mainly by snout vent length, tail length, and the number of ventral and subcaudal scales. In the BG-PCA scatter plot (Figure 8-5), the *nigrofasciatus* OTU shows a substantial overlap with the eastern OTU. One of the specimens of the novum OTU lies in between all other OTUs, whereas the other specimen lies outside of

the range of the other OTUs. The first principal component explains 67.23% of the total variation and the second component 28.98%.

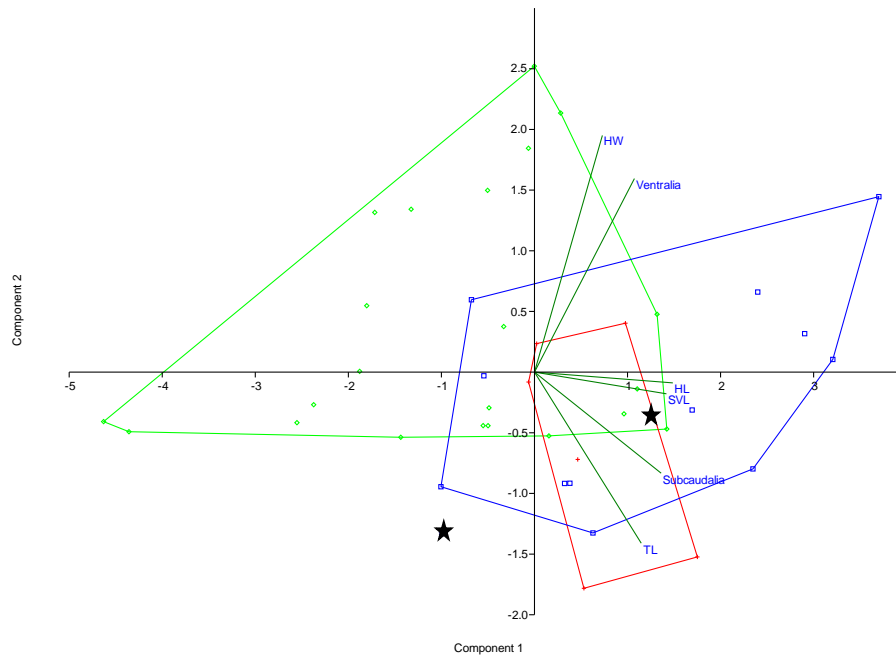


Figure 8-5. Scatter plot resulting from the between-group PCA on specimens of the eastern (blue), western (green), *nigrofasciatus* (red) and novum (star) OTUs. All variables and their contribution (factor loading) in OTU divergence are superimposed on the plots.

The MANOVA using characters that were normally distributed and showed a homogeneous variance (TL/TOL, HL/TOL, HW/TOL, Ven), reveals that only the eastern OTU is significantly different from the western OTU (Table 8-1). The ANCOVA and ANOVA with LSD post hoc pairwise test between the three OTUs reveals that, in the eastern OTU, tail length (TL) is significantly higher than western OTU and head width (HW) in the eastern OTU is significantly lower than western OTU. The npMANOVA reveals that the eastern, western and *nigrofasciatus* OTUs are significantly different from each other (Table 8-2). The novum OTU was excluded from the MANOVA, npMANOVA, ANOVA and ANCOVA because of the low number of samples in this OTU.

Table 8-1. Bonferroni-corrected *p* value, resulting from the multivariate analysis of variance (MANOVA) on metric and meristic characters of three OTUs of *Eirenis persicus*.

-	Western	<i>nigrofasciatus</i>	Eastern
Western	-	0.34	<0.05
<i>nigrofasciatus</i>	0.34	-	1.00
Eastern	<0.05	1.00	-

Table 8-2. Bonferroni-corrected *p* and *F* values resulting from the one way non-parametric multivariate analysis of variance (npMANOVA) on binary and categorical characters of three OTUs of *Eirenis persicus*. Significance level for *F* Test is 3.60. Significant *F* values are marked by **.

<i>P</i> value				<i>F</i> value		
-	Western	<i>nigrofasciatus</i>	Eastern	Western	<i>nigrofasciatus</i>	Eastern
Western	-	<0.05	<0.05	-	7.08**	20.65**
<i>nigrofasciatus</i>	<0.05	-	0.31	7.08**	-	2.38
Eastern	<0.05	0.31	-	20.65**	2.38	-

A Kruskal-Wallis analysis reveals that the number of subcaudal scales (Scd), the number of anterior and posterior dorsal scales (Sq.F, Sq.H) and the number of scales between the posterior chinshield (bet.po.chin) each are significantly different between the OTUs (Table 8-3).

Table 8-3. *p* value, resulting from the Kruskal-Wallis analysis on non-parametric characters of *Eirenis persicus* specimens.

	Pre.	Scd	Sq.F	Sq.H	Sup	inf	Lor	bet.l.inf	bet.po.chin	hind.par
Chi-Square	4.33	16.81	10.33	18.79	3.24	5.79	2.62	0.01	6.97	3.99
df	2	2	2	2	2	2	2	2	2	2
Sig.	0.11	0.00	0.01	0.00	0.20	0.06	0.27	1.00	0.05	0.14

8-3-3- Intra-OTU variation

The separate analysis of within-group variation in the eastern OTU showed that it is composed of two phenotypes. Specimens of Pakistan diverge from those of eastern Iran and Turkmenistan, mainly in the number of ventral and subcaudal scales (Figure 8-6). The first three components cover 92.53% of the total variation (Table 8-3). In the analysis on the western OTU, the first three components cover 94.23% of total variation (Table 8-3). The scatter plots (Figure 8-7) reveal that also here two groups can be distinguished: a Turkish group and an Iranian group. These groups differ mainly in snout vent length, tail length and the number of subcaudal scales.

Table 8-3. Principal components covering 95% of total variation in specimens of eastern OTU.

Eastern OTU		Western OTU	
PC	% variance	PC	% variance
1	57.18	1	53.30
2	20.10	2	30
3	15.25	3	10.93

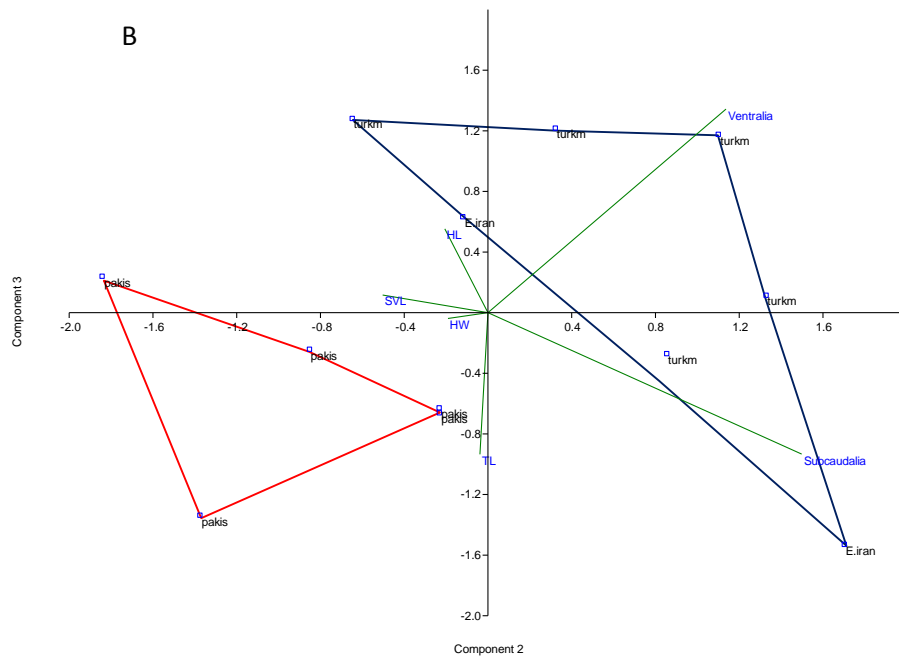
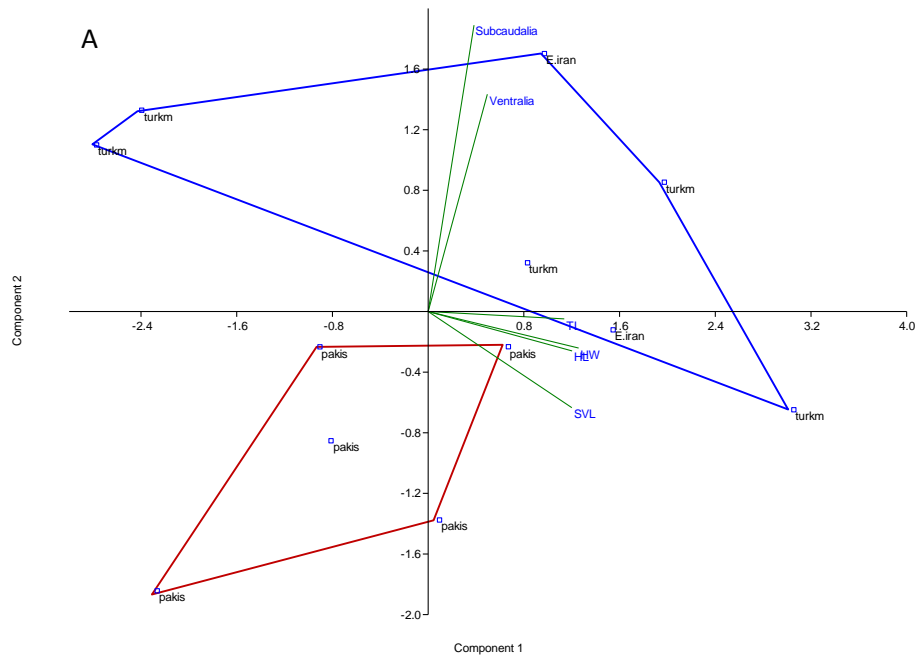


Figure 8-6. Scatter plot resulting from the within-group PCA on specimens of the eastern OTU. A: component 1 vs. 2; B: component 2 vs. 3. Red line: specimens of Pakistan. Blue: specimens of eastern Iran and Turkmenistan.

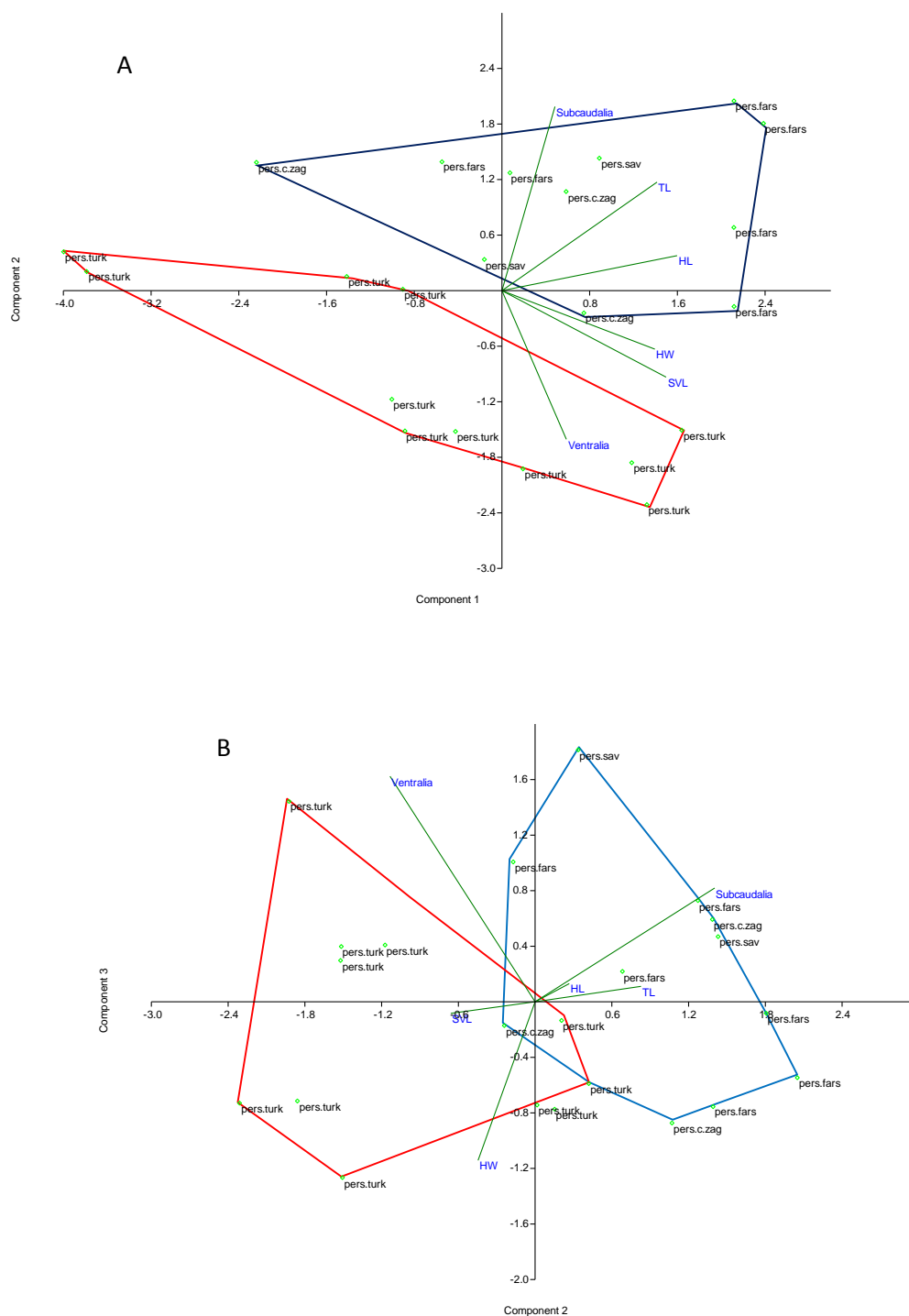


Figure 8-7. Scatter plot resulting from the within-group PCA on specimens of the western OTU. A: component 1 vs. 2; B: component 2 vs. 3. Blue: specimens of Iran. Red: specimens of Turkey.

Table 8-4. Descriptive statistics (above: mean \pm S.E., below: range) of the metric, meristic and categorical characters, partitioned into all groups (OTUs and groupings within OTUs) within *Eirenis persicus*. Definition of characters are in Table 2-1. The ratio characters were multiplied by 100.

Metric and meristic characters in male specimens

	persicus_Turkey	persicus_Iran	Novum	nigrofasciatus	E. Iran+Turkm	Pakistan
Number	5	6	2	3	3	4
TL/OL	15.3 ± 0.96 13.43 - 18.01	23.83 ± 1.11 21.93 - 29.02	25.08 ± 0.26 24.82 - 25.34	26.08 ± 2.26 21.57 - 28.49	26.16 ± 1.01 25 - 28.18	23.2 ± 1.16 19.74 - 24.84
HL/TOL	2.29 ± 0.08 2 - 2.41	2.53 ± 0.11 2.17 - 2.84	2.38 ± 0.32 2.06 - 2.7	2.3 ± 0.14 2.02 - 2.44	2.66 ± 0.46 2.17 - 3.58	2.34 ± 0.11 2.03 - 2.55
HW/TOL	1.74 ± 0.06 1.65 - 1.92	1.66 ± 0.05 1.51 - 1.83	1.46 ± 0.05 1.41 - 1.51	1.32 ± 0.04 1.25 - 1.39	1.59 ± 0.24 1.33 - 2.06	1.39 ± 0.03 1.35 - 1.48
Ven	203 ± 3.9 193 - 213	196.5 ± 2.08 190 - 203	198.5 ± 3.5 195 - 202	196.67 ± 2.03 193 - 200	217.67 ± 3.18 214 - 224	200.75 ± 2.87 194 - 208
Scd	49.6 ± 1.47 46 - 54	75.17 ± 2.89 64 - 83	77.5 ± 5.5 72 - 83	84 ± 8.74 67 - 96	95.67 ± 4.26 90 - 104	78 ± 4.38 66 - 85

Metric and meristic characters in female specimens

	persicus_Turkey	persicus_Iran	nigrofasciatus	E. Iran+Turkm	Pakistan
Number	4	5	3	4	1
TL/TOL	14.3 ± 0.36 13.22 - 14.79	20.39 ± 0.6 18.92 - 22.6	19.8 ± 0.49 19.02 - 20.69	20.73 ± 0.84 18.72 - 22.81	25.94 ± 0.0 25.94 - 25.94
HL/TOL	2.22 ± 0.02 2.19 - 2.25	2.79 ± 0.22 2.39 - 3.64	2.44 ± 0.09 2.33 - 2.62	2.62 ± 0.33 1.98 - 3.55	2.45 ± 0.0 2.45 - 2.45
HW/TOL	5.06 ± 0.48 4.18 - 6.4	4.54 ± 0.33 3.6 - 5.1	4.8 ± 0.26 4.4 - 5.3	5.15 ± 0.52 4.0 - 6.5	5.3 ± 0.0 5.3 - 5.3
Ven	214.75 ± 3.22 209 - 224	206.6 ± 3.03 198 - 214	210 ± 0.58 209 - 211	226.25 ± 2.29 223 - 233	184 ± 0.0 184 - 184
Scd	47.75 ± 2.1 42 - 52	66.8 ± 3.71 54 - 77	70.67 ± 1.76 68 - 74	81.25 ± 0.75 80 - 83	81 ± 0.0 81 - 81

Categorical characters in both sexes

	persicus_Turkey	persicus_Iran	Novum	nigrofasciatus	E. Iran + Turkm	Pakistan
Number	9	9	2	6	7	5
Pre	2.11 ± 0.2 1 - 3	2.11 ± 0.31 1 - 3	2 ± 1 1 - 3	2.17 ± 0.31 1 - 3	3 ± 0.31 2 - 4	2.4 ± 0.4 1 - 3
Sq.F	15 ± 0 15 - 15	15 ± 0 15 - 15	15 ± 0 15 - 15	15 ± 0 15 - 15	15 ± 0 15 - 15	15 ± 0 15 - 115
Sq.H	15.11 ± 0.11 15 - 16	14.82 ± 0.18 13 - 15	14.5 ± 0.5 14 - 15	13 ± 0.26 12 - 14	14.43 ± 0.37 13 - 15	13.8 ± 0.49 13 - 15
Sup	14.11 ± 0.11 14 - 15	14.22 ± 0.22 14 - 16	13.5 ± 0.5 13 - 14	14 ± 0 14	14 ± 0 14	13.5 ± 0.5 13 - 14
inf	14.44 ± 0.18 14 - 15	15.89 ± 0.11 15 - 16	16 ± 0 16 - 16	15.67 ± 0.21 15 - 16	15.86 ± 0.14 15 - 16	16 ± 0 16 - 16
Lor	0.33 ± 0.17 0 - 1	0 0	0 0	0 0	0.29 ± 0.18 0 - 1	0.4 ± 0.24 0 - 1
bet.l.inf	10.78 ± 0.22 10 - 12	9.44 ± 0.29 8 - 10	10 ± 1 9 - 11	10 ± 0.32 9 - 11	10.71 ± 0.29 10 - 12	9 ± 0.58 8 - 10
bet.po.chin	1 ± 0 1	0.45 ± 0.16 0 - 1	0 0	0 0	0.43 ± 0.2 0 - 1	0 0
hind.par	9.89 ± 0.31 8 - 11	10.64 ± 0.28 9 - 12	11 ± 0 11 - 11	10 ± 0.32 9 - 11	11.43 ± 0.2 11 - 12	10 ± 0.45 9 - 11

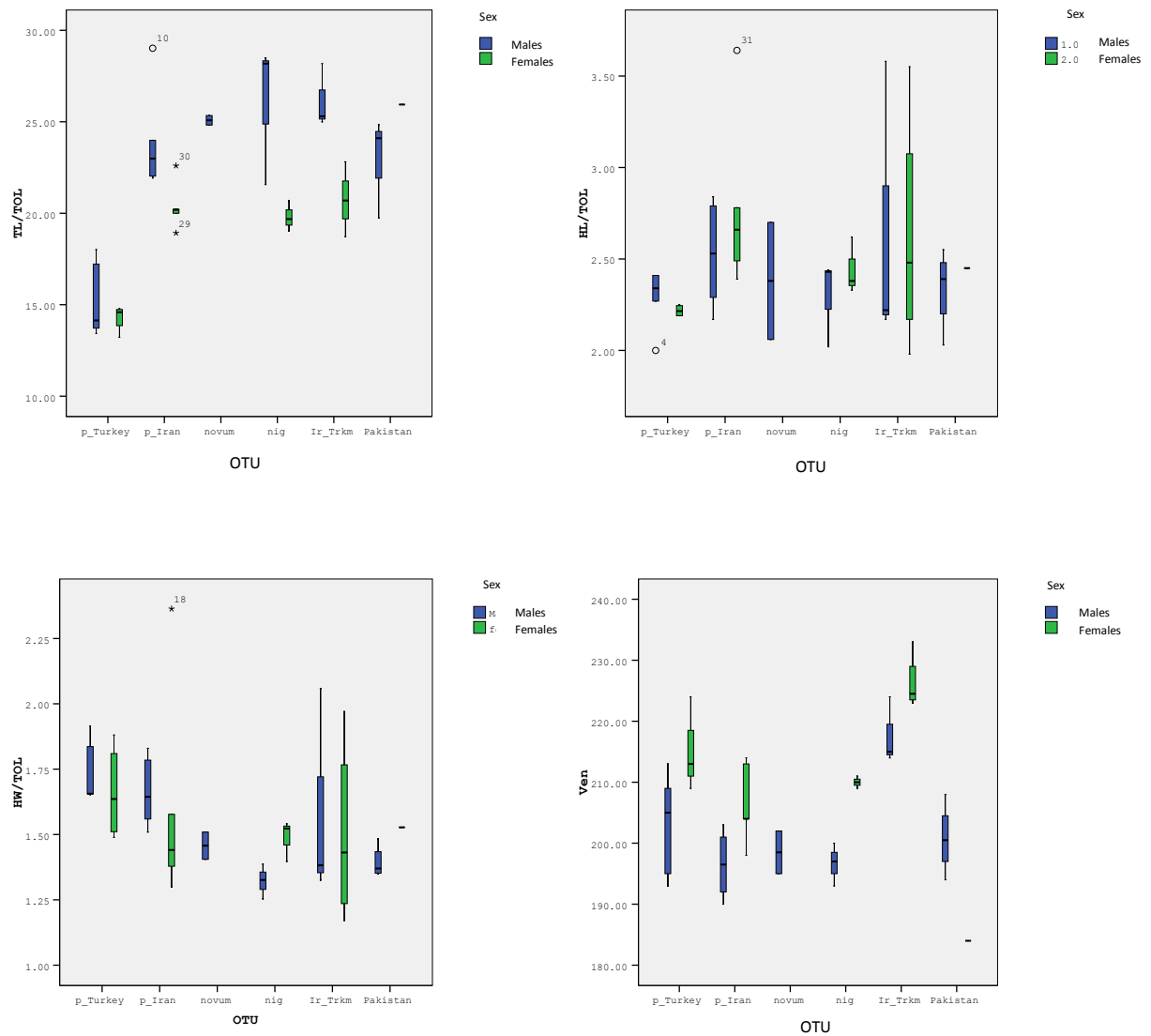


Figure 8-8. Box plots showing the minimum, first quartile, median, third quartile, and maximum for the morphological characters, partitioned into all groups (OTUs and groupings within OTUs) within *Eirenis persicus*. P-Turkey: *Eirenis persicus* specimens from Turkey; p-Iran: *Eirenis persicus* specimens from Iran; novum: novum OTU; Ir-Trkm: *Eirenis persicus* specimens from eastern Iran and Turkmenistan; Pakistan: *Eirenis persicus* specimens from Pakistan. P-Turkey and p-Iran are sub-groupings within the western OTU resulting from the within-group PCA. Ir-Trkm and Pakistan are sub-groupings within the eastern OTU resulting from within-group PCA. The ratio characters were multiplied by 100. Continued on next page.

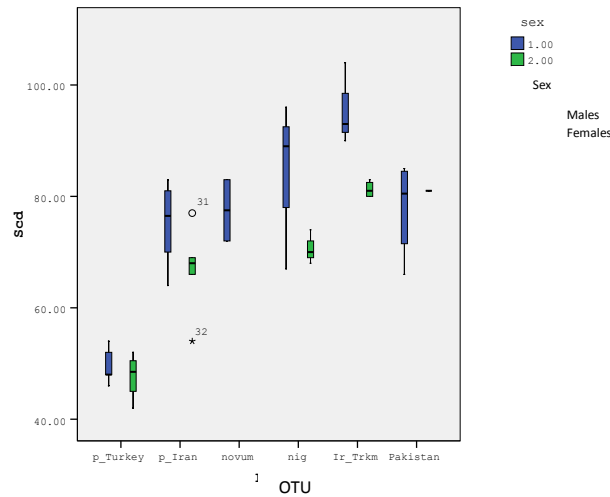


Figure 8-8. Continued. Caption in previous page.

8-3-4- Geometric morphometric analysis

The between-group PCA plot reveals that the western OTU diverges from both the eastern and *nigrofasciatus* OTUs (Figure 8-9). The deformation grids, reflecting shape variation within and across OTUs, (Figure 8-10), show that this divergence mainly results from the variation in the shape of the internasal, prefrontal, supraocular and parietal scales. The *nigrofasciatus* OTU shows a substantial overlap with the eastern OTU. Both specimens of the novum OTU lie outside the range of the eastern, western and *nigrofasciatus* OTUs. The npMANOVA reveals that the observed difference between the OTUs is not significant. The analysis repeated again using landmarks type 1 (Figure 8-3), but again the npMANOVA reveals that the observed difference between the OTUs is not significant.

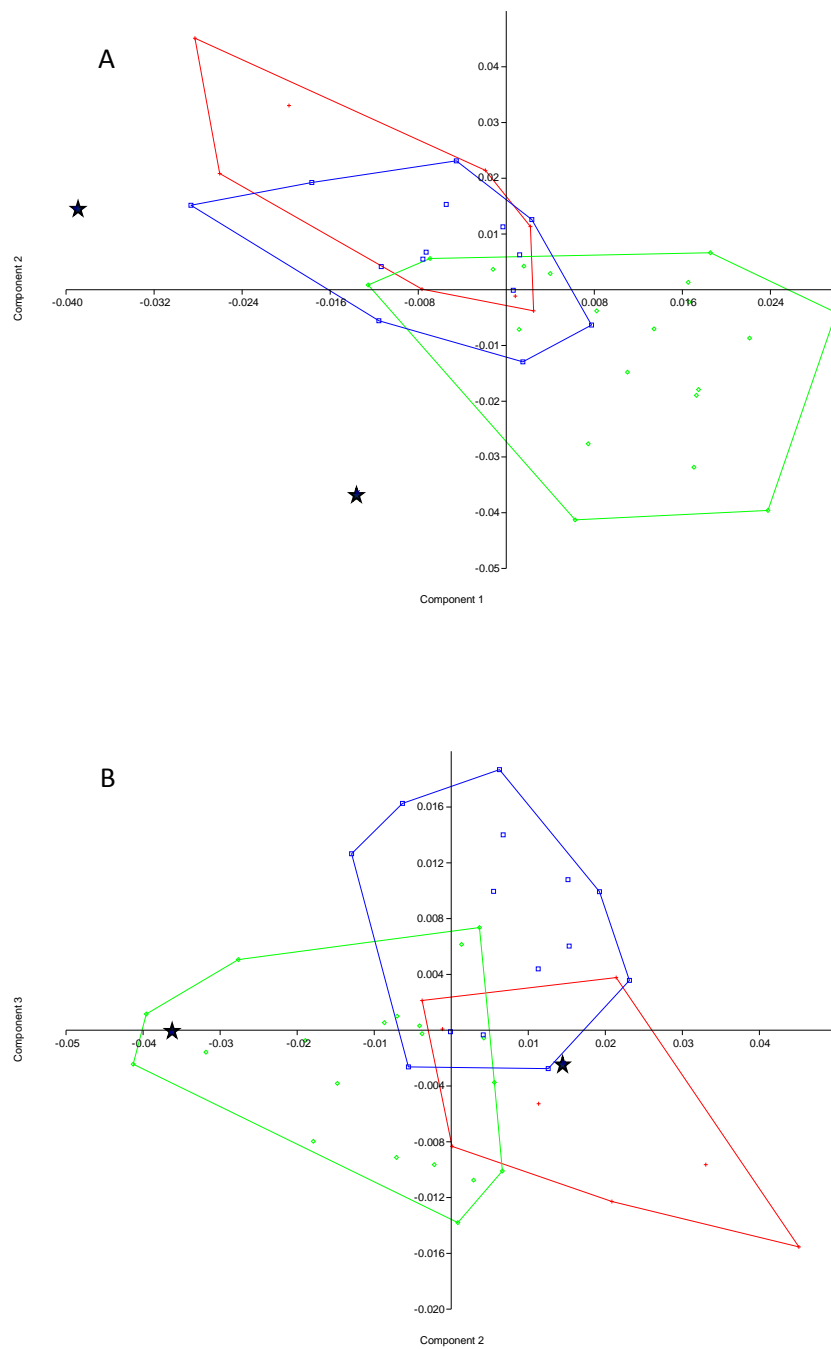


Figure 8-9. Scatter plot resulting from the between-group PCA on geometric data of the dorsal head scales. Eastern OTU (blue), western (green), novum (blue star) *nigrofasciatus* (red). A: component 1 vs. 2; B: component 2 vs. 3. The first component covers 53.29%, the second component 39.72% and the third component 6.98% of the total variation.

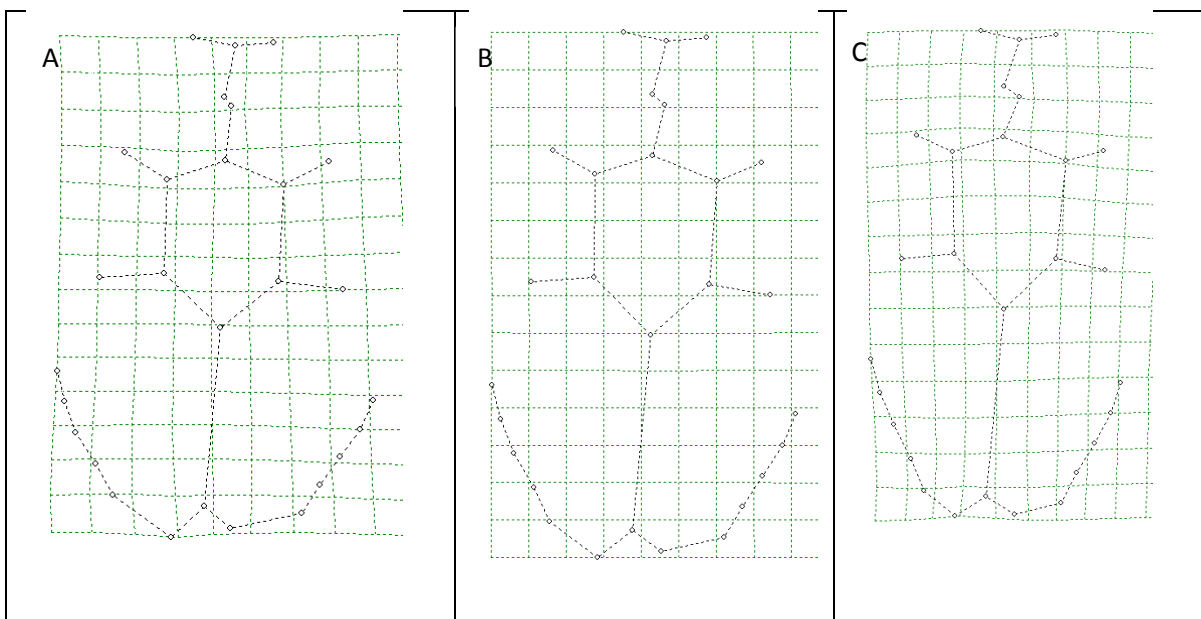
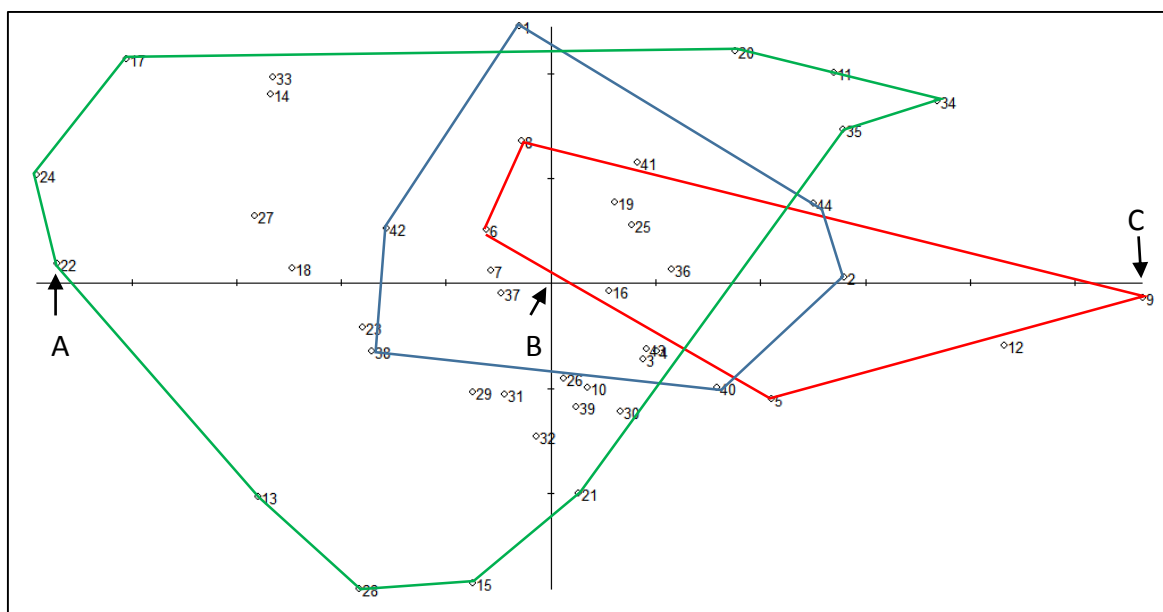


Figure 8-10. Deformation grids showing shape variation within and across OTUs. Eastern OTU (blue), western OTU (green), novum OTU (blue star) *nigrofasciatus* OTU (red).

8-3-5- Molecular analysis

In the molecular analysis, the Bayesian tree of cytochrome *b*, 16S rRNA and c-mos sequences (Figure 8-12) reveals that the whole *persicus* complex is supported very well as a monophyletic unit (posterior probabilities: 1). Nevertheless, within this complex there is a remarkable divergence, with all substructuring being supported very well also. Although few samples of the *Eirenis persicus* group was used in this analysis (no specimen of the novum OTU and north western Pakistan were included and only one specimen from eastern Iran (eastern OTU) was used. The Bayesian inference reveals that the *Eirenis persicus* clade has a basal branch, resulting in a clade comprising the *Eirenis persicus* specimens of western Iran and Turkey, and another clade represented by a specimen of the *walteri* morph from southeastern Iran and a specimen of the *nigrofasciatus* morph.

In the clade comprising the specimens of the western OTU, a divergence between the *Eirenis persicus* population of Iran and that of Turkey is observed. Also, in the clade of the eastern OTU, the *nigrofasciatus* morph is grouped with the *walteri* morph from the southeastern Iran. In this clade, there is a remarkable molecular distance between the *nigrofasciatus* and *walteri* morphs (Table 8-6).

Pairwise uncorrected genetic divergences of the cytochrome *b* gene (Table 8-6) give a detailed quantitative data about the divergence in the *Eirenis persicus* group.



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Table 8-6. Pairwise uncorrected genetic divergences of the cytochrome b gene in selected species of genus *Hierophis* and *Eirenis*, including *Eirenis persicus* group.

A	<i>H-viridiflavus</i>	A	B	C	D	E	F	G	H	I	J	K	L	M	N
B	<i>H-spinalis</i>	13.063													
C	<i>E-punctatolineatus</i>	14.054	14.505												
D	<i>E-coronelloides</i>	11.712	12.252	11.081											
E	<i>E-rothi</i>	12.703	14.775	12.432	11.532										
F	<i>E-decemlineata</i>	11.802	12.072	11.532	10.991	11.892									
G	<i>E-barani barani</i>	13.063	13.784	10.270	11.081	12.072	10.811								
H	<i>E-levantinus</i>	13.514	13.604	11.802	10.721	11.441	10.360	10.450							
I	<i>E-collaris</i>	12.162	12.432	11.171	10.18	11.081	9.8198	10.991	10.270						
J	<i>E-modestus</i>	12.523	13.063	13.063	10.270	12.703	10.811	11.622	11.441	11.622					
K	<i>E-persicus Turkey</i>	13.874	13.423	13.784	13.243	13.784	12.162	13.964	12.072	13.514	12.793				
L	<i>E. persicus walteri morph</i>	12.973	13.153	14.505	12.613	12.072	12.162	13.604	13.063	12.432	14.144	9.2793			
M	<i>E. p. nigrofasciatus morph</i>	12.613	13.243	14.595	12.703	12.703	12.432	13.243	12.432	11.802	13.694	10.360	7.2973		
N	<i>E. p. persicus morphTurkey</i>	12.342	13.784	14.685	12.432	13.514	11.892	14.685	12.252	12.793	12.973	5.8559	9.3694	10.270	
O	<i>E. p. persicus morphTurkey</i>	12.252	13.694	14.775	12.342	13.604	11.982	14.775	12.342	12.883	12.883	5.7658	9.4595	10.360	0.090

8-4- Discussion

Traditional biometry, geometric morphometrics and molecular data have shown the existence of distinctive groupings within the *Eirenis persicus* complex. Biometric data revealed the divergence between specimens of the western and eastern OTUs. This divergence in parametric and non-parametric data was significant. Based on the geometric data, the eastern and western OTUs showed to be different (but not significant) in the shape of their snout and the shape of the frontal and parietal scales. Molecular data support the morphological data and show a more than 10% DNA divergence between the eastern and western OTUs. The comparison of the DNA divergence in the *cytb* gene between the different valid species of the genus *Eirenis* (Table 8-6) reveals that a 10.2 - 11.8% DNA divergence is a reliable distance for the delimitation of the species in the genus *Eirenis*. Because the type locality of *Eirenis persicus* (Anderson, 1987) is located in the southwestern of Iran, present data indicate that specimens that belong to the eastern OTU (specimens from eastern Iran and Turkmenistan) are sufficiently different from those of topotypic *Eirenis persicus* (Anderson, 1987) to consider the eastern OTU as being a different species. In contrast to the *Eirenis persicus* specimens of western Iran and Turkey, that only have the *persicus* color pattern, the eastern group comprises specimens with both the *persicus* and *walteri* patterns. The oldest available synonym for this eastern group is *Pseudocyclophis* (= *Eirenis*) *walteri*

Boettger, 1888. We suggest to classify *Eirenis* cf. *persicus* specimens of eastern Iran, Turkmenistan and Pakistan, as *Eirenis walteri* (Boettger, 1888).

Biometric data confirm the divergence between *Eirenis persicus* populations of Iran and of Turkey, although not being a significant. Molecular data also show that populations of Iran and Turkey have about 6% DNA divergence in the *cytb* gene. This data indicates that *Eirenis persicus* specimens of Iran and Turkey are different at the subspecific level.

In all morphological and molecular analyses, specimens with a *nigrofasciatus* pattern were similar to specimens of the eastern OTU. Specimens of the *nigrofasciatus* OTU are significantly different from specimens of the western OTU. This significant difference was observed in the higher number of subcaudal scales. In the biometric analysis using selected head distances data, the specimens with the *nigrofasciatus* color pattern were different from the *Eirenis persicus* specimens of western Iran and Turkey. Molecular data confirmed this divergence and showed a DNA divergence of 10.2 – 10.3% between them. Specimens with the *nigrofasciatus* pattern were significantly different from those of the eastern OTU (eastern Iran and Turkmenistan) in the non-parametric analysis too. These specimens have a 7.2% DNA divergence from eastern OTU specimens, which is lower than the level of species delimitation. As a result, we conclude that specimens with the *nigrofasciatus* pattern are closely related to those of the eastern Iran, Turkmenistan and Pakistan (*Eirenis walteri*), but should be considered to belong to a different subspecific, *Eirenis walteri nigrofasciatus*.

Biometric data showed that specimens of northwestern Pakistan differ from those of eastern Iran and Turkmenistan in the number of ventral and subcaudal scales and in the ratios of tail length over total length. This could not be tested with molecular data yet, and further data is needed to clarify the taxonomy of these specimens.

The specimens of novum OTU didn't show any affinity to any of the identified groups within *Eirenis persicus*. Since only two specimens of this color morph were examined, we were not able to reliably compare them with other populations of the *Eirenis persicus* species complex. But the fact that these specimens are not distinctly different from other taxa in the *Eirenis persicus* species complex, suggests that they are not a different taxon. Probably these specimens just represent a color variation of the *persicus* or *nigrofasciatus* pattern. Further

investigations, including molecular studies, are needed to clarify the true nature of this new color morph.

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CHAPTER 9

General Discussion

9-1- Taxonomy of the examined species of the genera *Eirenis*, *Montivipera* and *Natrix* in Iran.

For a proper taxonomy, the potential of a character to clarify a taxonomic problem has to be carefully evaluated. Also characters should be evaluated taking into account the evolutionary forces driving the speciation process (Padial *et al.*, 2010). Adaptation to different ecosystems that lead to speciation can't simply distinguish between snakes populations. Considerable amount of intra population variation in morphometric and meristic traits have been documented in snakes. Forsman (1991) observed intraspecific variation in head length among *Vipera berus* populations, which are determined by the size of the consumed voles. Many studies confirmed that there is a significant impact of temperature on the number of ventral scales in newborn snakes, with higher temperatures increasing scale numbers (e.g.; Lourdais *et al.*, 2004; Osypka and Arnold, 2000). Similar results were inferred from climate-correlated geographic shifts in scale counts (Klauber 1941).

In this thesis, the taxonomy of some snake genera was mainly investigated using morphological data. In phenotypic species criteria, the most widely applied morphological method of delimiting species is to base the species status on the presence of fixed or non-overlapping morphological character differences between geographical samples (Wiens and Servedio, 2000). Alternatively, multivariate analysis of phenotypes can be used to identify groupings (phenetic clusters), which are considered species in the absence of intermediates (Sokal and Crovello, 1970). In the case that morphological data was not enough for decision about taxonomy of a species complex (e.g. *Eirenis persicus* species complex), a phylogenetic analysis based on DNA sequences were used for species delimitations. Lineage-based species concepts recognize species on the basis of monophyly of gene genealogies (for more details, see 1-1-4). However, the monophyly criterion is unlikely to lead to reliable species

delimitation always (see Sanders *et al.*, 2006). So mitochondrial pairwise sequence differences are also used to delimit species (see Johns and Avise, 1998).

Taxonomy is directly affected by the adopted concept of species. Different sources of data that fit with different concepts of species, were used in the taxonomic studies in this thesis. In this thesis, following De Queiroz (2007,) and Padial *et al.*, (2010) the integration by cumulation approach was used to distinguish different species. So, all of the properties of species criteria (intrinsic reproductive isolation, diagnosability and monophyly) provided us evidence for lineage separation. This method is more appropriate for the purpose of this study, because we had different set of taxonomical characters for the examined snakes. Integration by cumulation approach allowed us to focus on the most available set of taxonomic characters for each group of snakes. This approach doesn't bind species delimitation to all species properties (as is required for the integration by congruence approach). Indeed, usage of the integration by cumulation approach superficially leads to the traditional approach of morphological taxonomy before the massive incorporation of other characters. We tried to incorporate all possible species properties (including intrinsic reproductive isolation, diagnosability and monophyly) and thus all possible lines of evidence for final taxonomic decisions. In the following, we discuss taxonomic conclusions of the examined species of the genera *Eirenis*, *Montivipera* and *Natrix* in Iran.

9-1-1- Taxonomy of *Montivipera raddei* species complex

Although color pattern was used as one of the distinguishing traits by Nilson and Andren (1984; 1985; 1986), studies explained in chapter three showed that color patterns in the *Montivipera raddei* species complex are not independent character from environmental factors and are not reliable characters for taxonomy. Color patterns are polymorphic within populations of the *Montivipera raddei* species complex, but with color morphs occurring at different frequencies across the different taxa.

Phylogenetic studies based on mitochondrial DNA sequences revealed that the diversification and dispersal of mountain vipers of genus *Montivipera* was triggered by allopatric speciation during the Plio-Pleistocene mountain formation periods and both geographic and altitude isolations in mountainous systems were involved in the speciation of these mountain vipers (Stümpel *et al.*, in press).

Montivipera latifii and *Montivipera kuhrangica* are both species at the extreme eastern and southern distribution range of the *Montivipera raddei* species complex. Based on biochemical data, Hermann and Joger (1977) suggest to consider *M. latifii* as a subspecies of *M. raddei*. Also Joger (pers. comm.) considered *M. kuhrangica* as a subspecies of *M. raddei*. But detailed molecular studies (Stümpel and Joger, 2009) revealed that *M. latifii* is a separate lineage within the *M. raddei* species complex. Multivariate studies (see chapter three) also confirm that *M. latifii* is significantly different from *M. raddei* when combining morphometric and meristic characters. This difference is not statistically confirmed between *M. kuhrangica* and *M. raddei*, but *M. kuhrangica* shows some unique morphological characters in the number of head scales, which separate it from *M. raddei*. Although the sample size for the description of *M. kuhrangica* was small, the unique morphological character in head scales and isolated distribution provided strong support for the delimitation of this species. In addition to the description of *Montivipera kuhrangica*, the molecular phylogenetic studies based on mitochondrial DNA sequences (Stümpel *et al.*, in press) confirmed that this species forms an independent lineage within the *M. raddei* species complex and is thus a valid species from phylogenetic point of view too.

The taxonomic statue of the Zanjan valley viper is more complicated than that of other populations in the *M. raddei* species complex. The difference of this population with *M. raddei* is not as much as the difference of *M. latifii* or *M. kuhrangica* with *M. raddei*, not for the morphological data, nor the molecular data. From a phylogenetic point of view, the Zanjan valley viper is not monophyletic and should thus be considered a synonyme of *M. raddei* (Stümpel, pers. comm.). However, this study did reveal that this isolated population have certain, significantly different morphological features with other populations of *M. raddei*. Since the Zanjan valley population is an isolated population and morphologically distinguishable from other populations of *M. raddei*, it is a good candidate for subspecific classification.

9-1-2- Taxonomy of *Natrix tessellata*

Natrix tessellata has a wide distribution range, from central Europe to China (see Mebert 2011a, Ananjeva *et al.* 2005, Szczerbak 2003), and in this study only a small part of the species distribution range was studied. The possible reason for observing two significantly different populations of dice snake in a limited geographic range (northern and western mountains of

Iran) is that, mountains of Iran bear the most basal radiations within the *Natrix tessellata* lineage, are adapted to their local environment for a long time, while most of distribution range of the species in Europe and central Asia were occupied by the recent radiation, after the glaciation era (see Guicking *et al.* 2006). For a proper taxonomic decision, examination of enough specimens from around the distribution range of the species is essential. So taxonomic conclusion about significantly different populations of *Natrix tessellata* in the northern mountains of Iran and southern Zagros can't be made now and it postponed until enough specimens over the distribution range of this species are being examined.

9-1-3- Taxonomy of examined species of genus *Eirenis*

To evaluate the taxonomy of dwarf snakes of the genus *Eirenis*, we need to take a brief look at the evolution of the genus and the evolution of morphological traits, used in the taxonomy of these snakes. During the last two decades, various studies have revealed that dwarf snakes of the genus *Eirenis* (they are mainly less than 50 cm, see Schmidtler and Schmidtler, 1978) have evolved from big snakes of the genus *Hierophis* (adults typically being more than one meter) (Nagy *et al.*, 2003; Schätti 1988b; Schätti and Utiger, 2001; Schmidtler, 1993). This dwarfing in size has been coupled to a change in life style. While *Hierophis* snakes are surface dwelling, *Eirenis* snakes mainly have a cryptozoic lifestyle. An exception is *Eirenis punctatolineatus* which is mainly a surface dweller (Terent'ev and Chernov, 1965).

The most common effect of miniaturization on the morphology is reduction and structural simplification (Hanken and Wake, 1993). Snakes display few external diagnostic characters in the body and tail and most of morphological variation is thus observed in their head (see Thorpe, 1975). Compared to *Hierophis* snakes, dwarf snakes of the genus *Eirenis* have more simplified head scales and their head is covered by fewer number of scales, e.g. reduced number of temporal, preocular and labial scales (see Mahlow *et al.*, 2023; Gruber, 1989). So, differentiation in the head scales can be a very useful feature in species delimitations in *Eirenis* snakes. Because of this morphological simplification, when traditional morphological approaches are not able to properly quantify the patterns of morphological variation within and between populations, geometric morphometrics may prove to be a more powerful approach to reveal even subtle morphological variation in head scales of this snakes.

Molecular phylogenetic studies (Nagy *et al.*, 2003; Rajabizadeh *et al.*, unpublished data) showed that *Eirenis* snakes are an early radiation, with an extensive speciation that took place

in a limited geographic range. In this genus, molecular differentiation rates are generally higher than morphological differentiation rates (Nagy *et al.*, 2003; Rajabizadeh *et al.*, unpublished data). This implies that every taxonomic decision based on molecular data only should be done carefully, in order to avoid overestimation of species numbers. In many herpetological publications, mitochondrial DNA divergence ranging between 1.6% and 6.2%, are often used to delimit snake species (see Sanders *et al.*, 2006). In this study (see chapter eight), a comparison of pairwise genetic divergences of the cytochrome *b* gene between the confirmed *Eirenis* species revealed a reliable average of 10% DNA divergence, thus supporting the suggested species delimitations.

9-1-3-1- Taxonomy of *Eirenis medus* species complex

New specimens of *Eirenis medus*, collected in the course of this research from the northern and western mountains of Iran, reveal that the *Eirenis medus* species complex has a wide distribution range, from the type locality of *Eirenis medus* in the Kopet Dag Mountains (at the Iran and Turkmenistan border), through northern and western mountains of Iran, up to southern Zagros. Populations of Hamedan, Isfahan and Kerman all differed in their morphology from the population of the type locality, with that of Kerman showing the largest difference. At least some of this variation, like ratio of tail length over total length and number of subcaudal scales, showed a clinal variation from the northern mountains of the Iranian localities, to Isfahan and Kerman. Population of Kerman can be distinguished from all other populations of the *Eirenis medus* species complex in having deeper than long loreal scales. Although only two specimens from Kerman population is in hand and completed analyses on molecular data on this species complex is not available yet (Rajabizadeh *et al.*, unpublished data), assigning the Kerman population of the *Eirenis medus* species complex to a distinct species, based on unique morphological characters, is considered to be justified. In the other populations (Hamedan and Isfahan), there is no such a distinguishable morphological character, so any taxonomic decision about these populations must be postponed until the collection of more specimens and completion of these molecular studies. Although Mahlow *et al.* (2013) didn't accept the validity of *Eirenis kermanensis*, recently they changed their idea and accepted the validity of this species too (pers. comm.). Actually this kind of discussions are raised because of the low sample size of the new species and the doubt about the intra population variation of it. There are examples of the rare new species, described based on a

single specimens that still are considered as a valid species (e.g. *Eirenis africana* and *Eirenis rechingeri*). All of these species are very rare and are distinguished by the unique morphological characters. Since *Eirenis kermanensis* is rare (In several field expedition in the type locality only two specimens were collected), unfortunately it was not possible to assess the intra-population variation of it. From the biogeographic point of view, available data show that Kerman population is isolated from the other populations of *Eirenis medus* species complex. Respect to isolation of Kerman population and occurrence of the unique morphological characters in these specimens, it is reasonable to conclude that this population has diverged from the *Eirenis medus* by speciation, although there is not enough supplementary data of its intra-population variation.

9-1-3-2- Taxonomy of *Eirenis punctatolineatus*

The tail length and number of subcaudal scales, as well as difference in color pattern is significantly different between the southern Zagros populations of *Eirenis punctatolineatus* and the other populations in the northern mountains of Iran, southern Turkey and southern Armenia population. However, these metric and meristic characters are also correlated with the latitude, from north to south.

Molecular studies show that the northern and southern populations of *Eirenis punctatolineatus* have between 8 to 9% pairwise genetic divergences in their cytochrome *b* gene (Rajabizadeh *et al.*, unpublished data). Despite this amount of morphological and molecular differences, they cannot be considered as different species because of the morphological variation reflecting a clinal variation from north to south. Still, in this big cline, extended through Zagros mountain chain, the connection between the northern and southern populations of *Eirenis punctatolineatus* and normal gene flow between them, is being interrupted to some degree in the central Zagros region (see title 9-2). A transitional zone between the northern and southern phenotypes of *Eirenis punctatolineatus* is observed in the central Zagros. Diagnosable populations joined by a cline can be treated as subspecies (Patten and Unitt, 2002; Mayr and Ashlock 1969). Assigning the northern and southern populations of *Eirenis punctatolineatus* to different subspecies simply reflects the significant morphological and molecular difference between them, interruption of gene flow, as well as the connection between them by a transitional zone in the central Zagros.

9-1-3-3- Taxonomy of *Eirenis persicus* species complex

Taxonomy of *Eirenis persicus* species complex is much more complicated than the two other examined *Eirenis* species. This species complex has a wide distribution range in south western Asia and has different populations with different color morphs which taxonomy of them is not clear. Two significantly different groupings was revealed within this species complex, based on multivariate comparison of metric and meristic characters. Populations of the western Iranian plateau with the *persicus* color morph were significantly different from the other populations of this species complex. Although the morphometric and meristic traits incorporated in this analysis were significantly different, they were all partially overlapping traits. To explore about other possible groupings between populations of the *Eirenis persicus* species complex, and to ensure about the results obtained from traditional biometry, a geometric morphometrics analysis was applied. This analysis indeed confirmed the biometric results, but differences were not significant, and showed only overlapping morphological trait between the significant groupings. Still, the morphological data is not enough to support any argumentation about specific or subspecific diversification within this species complex. The molecular analysis revealed more details within these morphological groups. The Bayesian tree of the cytochrome *b*, 16S rRNA and c-mos sequences well confirmed two main groupings in the *Eirenis persicus* species complex, including a clade of populations of the western Iranian plateau with the *persicus* color morph and a sister clade of the other populations of this species complex. The Bayesian tree showed more substructuring within these sister clades too. About 10% pairwise genetic divergences of the cytochrome *b* gene was observed between the main clades in this analysis, providing supportive evidence for designating the two basic sister clades to separate species. It is advantageous to recognize the basal monophyletic groups as a species, but to also maintain the subspecific designations for the distinguishable groups within this lineages (Mulcahy, 2008). The substructuring within the clade of western Iranian plateau (with the *persicus* color morph) reflects two groups, one from southern Turkey and adjacent area in the north western Iran, and one from the southern Zagros mountain. These populations show difference in the number of ventral and subcaudal scales, as well as ratio of the tail length over total length. These groups are separated from each other by the central Zagros mountain. The substructuring within the sister clade is composed of two groups of populations too, a group representing the specimens with the *nigrofasciatus* morph, and another group for other populations in the eastern Iran and

Turkmenistan populations with the *persicus* and *walteri* pattern. These two groups are separated from each other by central Iran plains. Populations incorporated in the substructuring within each sister clade, are geographically separated, plus, molecularly and morphologically they are distinguishable and so are acceptable candidates for subspecies classification.

9-2- Biogeography of the examined species of genera *Eirenis*, *Montivipera* and *Natrix* in Iran.

Because of the geographic location of the northern and western mountains of Iran, being in the junction between different ecosystems, these mountains have affected the reptile fauna from the Mediterranean basin, central Asia and northern Africa. Snake lineages that had dispersed towards the northern and western mountains of Iran were influenced by the geological events and climatic oscillations of the area. Although geological events, like plate tectonics, affect the biodiversity at large scales (see Che *et al.*, 2010), Pleistocene climatic oscillations are also responsible for a large proportion of speciation (Avice and Walker, 1998). The greatest geological event which influenced snake biodiversity in the northern and western mountains of Iran is the formation of the Zagros mountain chain. The Alborz mountains are very old (formed about 210 Mya) and the last changes in these mountains were influenced by the uplifting of the Zagros mountains too (Berberian and King, 1981). This phenomenon that started with the closure of the new Tethys sea in about 19 Mya and ended about 12 Mya (Rogl, 1999), formed the land bridge between Africa and Eurasia on the one hand, and formed a new mountains chain, Zagros, on the other hand, opening up new habitats for snakes from both the African and Eurasian continents. During this process of snake dispersal into the Zagros and Alborz mountain chains, speciation occurred in these mountains through vicariance of the snake populations. The uplifting of the Zagros mountains and the Pleistocene climatic oscillations are the two most important factors explaining snake isolation throughout the northern and western mountains of Iran.

In this study, the divergence of snake populations from the southern Zagros from those from the northern Zagros was observed in *Natrix tessellata* and *Eirenis punctatolineatus*. In *Natrix tessellata*, these two populations were shown to be significantly different in their

morphological traits, suggesting that they may reflect local adaptation to their natural environment through isolation from each other for a long period of time. Following a divergence of *Natrix tessellata* from the common ancestor of the *Natrix* species, about 13 – 22 Mya, and dispersal into southwestern Asia and the Zagros mountain chain, the intraspecific divergence in *Natrix tessellata* between southwestern Asia, Europa and central Asia clades is estimated to have commenced between five to seven Mya (Guicking, *et al.*, 2006). Since the orogenesis in the northern and western mountains of Iran finished about 12 Mya, divergence between the northern and southern populations in *Natrix tessellata* can't refer to paleo-tectonic events and orogenesis of the Zagros mountain chain.



Figure 6-1. Map of Iran showing the highly elevated central Zagros (C) that separate the northern (N) and southern (S) Zagros mountains.

The *Eirenis punctatolineatus* populations from the southern Zagros also showed a divergence from its northern populations. Although divergences of the genus *Eirenis* go back to about 20 Mya (see Wüster *et al.*, 2008), respect to the molecular phylogeny of this genus *Eirenis* (Nagy *et al.*, 2003), divergence between the northern and southern Zagros populations of *Eirenis*

punctatolineatus can't be due to paleo-tectonics of the Zagros. It could as such be hypothesized that this divergence between the northern and southern populations in *Natrix tessellata* may refer to a more recent phenomenon, Pleistocene climate changes. Most of the speciation through isolation in these mountains will have been associated with the Pleistocene climate changes (Joger *et al.*, 2007; Veith *et al.*, 2003; Avise, 1998).

Results of this study reveal that *Eirenis punctatolineatus* inhabits the mountain sides. The hypothesis to explain the divergence of the northern and southern Zagros populations of *Natrix tessellata* and *Eirenis punctatolineatus* is that, through the Pleistocene climatic oscillations, climatic cooling could have shifted the habitat of these species to a lower altitude (because eco-zones shift to lower elevations), resulting in a reduced contact between the southern and northern populations (as the central Zagros chain became a relatively higher isolating barrier for these populations) (Figure 6-1 and 6-2). Subsequently, climatic warming shifted the range of acceptable ecological conditions back to higher altitudes again, allowing previously isolated populations to get into contact again. Because the Zagros mountain chain is adjacent to central and western Zagros plains, from east and west, there is no obvious connection between the northern and southern Zagros mountains, around the central Zagros.

The Central Zagros possibly also affected other populations of reptile species that are distributed across the Zagros chain. *Platyceps najadum*, *Hemorrhois ravergieri*, *Macrovipera lebetina* and *Malpolon insignitus* of the family Colubridae, are some example species that are distributed across the Zagros. *Platyceps najadum* has a subspecies, *Platyceps najadum schmidtleri*, that is distributed in the southern Zagros and reflects isolation of this population from the rest of *Platyceps najadum najadum* populations. Also, there are reports on the morphological difference of the southern Zagros populations of *Macrovipera lebetina* with the rest of the populations (see Mallow *et al.*, 2003). Further investigations are necessary to clarify whether the southern population of this species have been isolated from the rest of populations or not.

Central Alborz is also highly elevated, but there are little data available that show its role in the isolation of the snake populations in the eastern or western Alborz. In this study, no significant morphological differences were observed between the eastern and western Alborz populations of *Natrix tessellata*. As this dice snake is abundant in rivers and lagoons close to the Caspian Sea, it could be that the sea served as a corridor for its dispersal across the

northern side of the (western, central and eastern) Alborz mountains. The genus *Eirenis* doesn't show a wide distribution throughout the Alborz chain; also in this study only a few specimens were collected that originated from the western, central and eastern Alborz mountains. It could be interesting to compare this information with that of other widely distributed snakes in the northern and western mountains of Iran, such as *Hemorrhoids ravergeri*, to confirm this hypothesis that in general, snake populations from the western and eastern Alborz are significantly different, or not.

The Central Zagros not only isolates the southern Zagros from the north Zagros, it is also, itself, isolated from both the northern and the southern Zagros. This altitude isolation of the Central Zagros compared to the north and south is also reflected in the biogeographical isolation of populations living in the north and south from those living in the central Zagros (Figure 6-2). This phenomenon wasn't observe in species with wide distribution ranges, but was rather observed in snakes with a more local distribution. *Montivipera kuhrangica* is an example of a local population being isolated in the highly elevated, central Zagros Mountains.

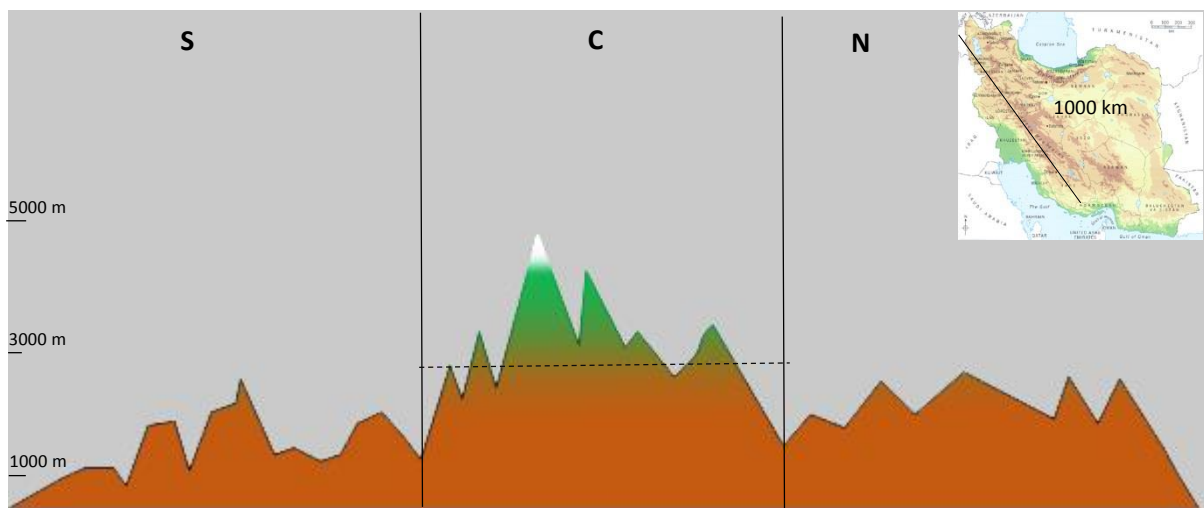


Figure 6-2. An approximate cross sectional profile of the Zagros mountain chain (1000 km length), generated based on geological data of Zagros mountain chain (Fisher, 1968), showing the southern (S), central (c) and northern (N) Zagros. The central Zagros is the highest part of the mountain range; it is not clear whether it is a geographic barrier for dispersal (similar to a mountain between two plains). Increased elevation of the central Zagros lead to different environmental conditions, such as temperature and precipitation ranges, resulting in different habitats than in the adjacent northern and southern Zagros (green gradient).

Vipers of the genus *Montivipera* were affected by Pleistocene climate changes. Climatic oscillations were the main driving force behind allopatric speciation in vipers of the genus *Montivipera* and isolation of *Montivipera raddei* species complex in the northern and western mountains of Iran (Stumpel *et al.*, in press). As discussed in chapter three, two populations of these vipers, i.e. *Montivipera kuhrangica* and *Montivipera latifii*, have been isolated in the central Zagros and central Alborz mountains, respectively, while various populations of *Montivipera raddei* occur throughout the northern Zagros, Azerbaijan and western Alborz mountains. During the warming periods of the Pleistocene climatic oscillations, the suitable habitat of these mountain vipers shifted to higher altitudes in the mountain, resulting in the isolation of the species in the mountains and patchy distribution of *Montivipera* taxa (Figure 6-3). Subsequently, climatic cooling shifted the range of these habitats back to lower altitudes. During the cooling period, the valley between the isolated mountains in northwestern Iran was again suitable habitat for the mountain vipers, probably allowing formerly isolated populations to get into contact again. (Figure 6-3). Thus populations of *Montivipera raddei* in the isolated mountains of northwestern Iran, isolated and re-contacted again throughout the Pleistocene. Molecular data (Stumpel and Joger, 2009) confirmed that the divergence between these populations is not as high as for different species. Through contacts between different populations, local adaptations in color pattern, were shared between different populations through gene flow, resulting in the increase color pattern variation in these populations. Because of phylogenetic niche conservatism (tendency of lineages to retain their niche-related traits through speciation) (Crisp and Cook, 2012), two isolated viper populations in the higher altitude of the central Alborz and the central Zagros mountains (including *M. kuhrangica* in central Zagros and *M. latifii* in central Alborz) were kept isolated after the Pleistocene climatic oscillation.

9-3- Cryptic snake biodiversity in Iran

Results of this study confirm the existence of cryptic biodiversity in snakes of the northern and western mountains of Iran. Between the studied species, the cases of cryptic biodiversity were observed in the *Eirenis persicus* species complex. Unpublished molecular data of

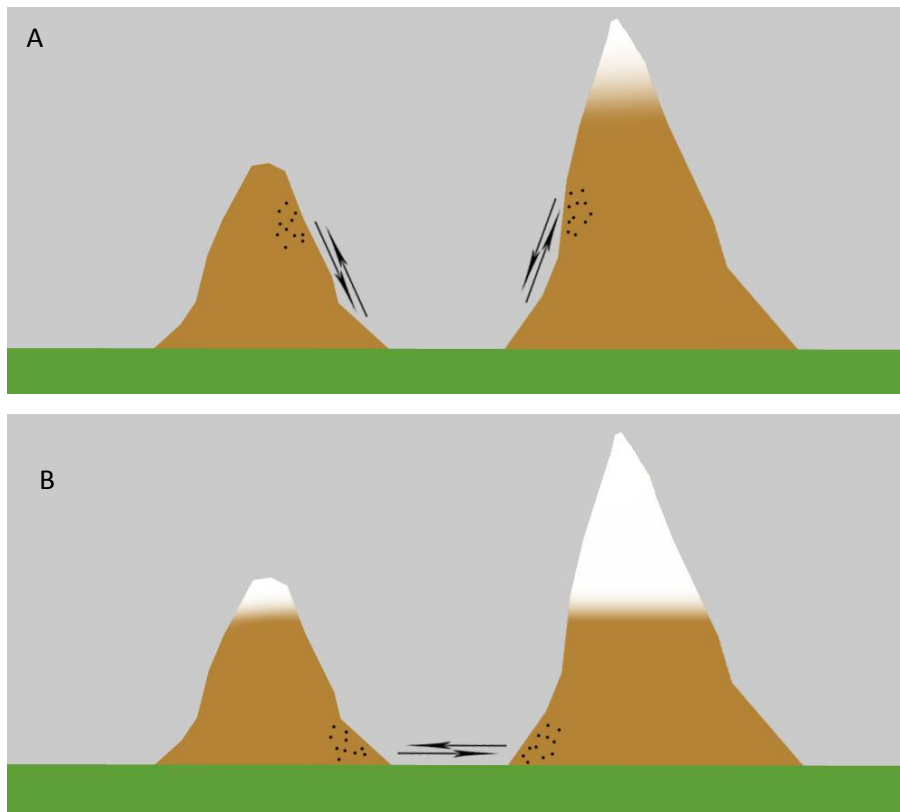


Figure 6-3. Distribution shift of mountain vipers of the genus *Montivipera* (dots) through warming (A) and cooling (B) periods of the Pleistocene. Arrows indicate the natural movement of the vipers across the home range.

M. Rajabizadeh and Z. Nagy confirm occurrence of cryptic biodiversity in other species of the genus *Eirenis* too. Although cryptic species only are the product of the recent speciation (see Elmer *et al.*, 2007), in the case of *Eirenis* snakes, rapid speciation may explain why morphological variation across the *Eirenis* species is not well correlated with species boundaries (see Nagy *et al.*, 2003). Because of overall morphological similarity between the species of this genus, two or more species were previously incorrectly classified as a single taxon. The *Eirenis persicus* species complex is an example of such an incorrect classification. Although traditional biometry failed to distinguish these cryptic species in the genus *Eirenis*, the use of molecular studies only can't resolve this problem. The rate of DNA divergence within the *Eirenis* species is relatively high (Nagy *et al.*, 2003). This higher rate of genetic divergence may result from a high rate of isolation in these small size snakes, within a small home range. Using only molecular studies to assess the biodiversity within the genus *Eirenis* may lead to an overestimation of species numbers. Present studies on the *Eirenis persicus* group revealed that, the combination of morphological analyses (including traditional

biometry and geometric morphometrics) and molecular studies, can give a more reliable result to allow a proper classification of the *Eirenis* snakes.

The geometric morphometric analyses can enhance the level of detail for a morphological comparison between populations, as it can detect subtle variations in shape between populations. One of the main general themes, explaining why morphological change might not be useful in discriminating species, is to be under selection that promotes morphological stasis (Bickford *et al.*, 2007). In this situation, morphological variation is very low. Still, geometric morphometrics are in such a way a powerful morphological technique, that they facilitate revealing very small, morphological variations that can improve the delimitation of the species based on morphological traits. As observed in *Eirenis persicus*, the reconstruction of shape variation visualized through deformity grids and the exploration of patterns of shape variation between populations can guide the researcher to the most informative part of the head scales, where components of shape variation between the populations may still be indicative for distinctive populational traits.

When assessing biodiversity, and especially cryptic biodiversity, attention must be paid to environmental factors. Part of the variation observed in the snake populations may thus reflect local adaptation of allopatric populations (of a single species) to the local environmental conditions. As was observed in the color pattern variation in the genus *Montivipera*, color pattern was related to the local environmental factors. The adaptive nature here could be found in the appearance of the snake color pattern with respect to the surrounding vegetation and substrate color. So, giving taxonomical significance to these characters would result in the recognition of local populations within a single species as different taxa and hence result in the overestimation of the biodiversity.

9-4- Importance of the new findings in this thesis

New findings in this research, shed more light on the biodiversity of the snakes of Iran. This data are especially important from a conservation point of view. Also these new findings help to improve the knowledge about phylogeny, biogeography and evolution of the studied snake's genera.

A recent modeling analysis of the habitat suitability of the *Montivipera raddei* species complex, using climatic variables (Kaboli *et al.*, in prep), reveals that potential habitats of

Montivipera in the central Zagros (including Kuhrang region) are very small and scattered. Small population sizes (inferred from less than 5 observed specimens in Kuhrang region during the last decade), low available potential habitats and occurrence of habitat destruction (at least caused by overgrazing in the Kuhrang region) all indicate that *Montivipera kuhrangica* is highly faced with a threat to extinction. Based on this data, applied to register this species in the IUCN red list of threatened species (submitted by Rajabizadeh, Nilson and Kami).

The new findings about inter population variation of *Natrix tessellata* in Iran are primary insights for taxonomic diversifications with this species. Recent molecular phylogenetic studies confirm a splitting of this broadly distributed species into several species (Joger, pers. comm.).

This study revealed the occurrence of several, isolated populations of dwarf snakes of the genus *Eirenis* along the Zagros mountain chain. One of this population is assigned to a new species, *Eirenis kermanensis*. This isolated population with limited geographic range is especially important for conservation matters.

This study sheds more light on the taxonomy of the *Eirenis persicus* species complex. Understanding the taxonomy of this species complex help us to improve the phylogeny of dwarf snakes of genus *Eirenis* and to have a better view about the evolution of dwarfing in this snakes.

9-5- Checklist of the Snakes in Northern and Western Mountains of Iran

In the present chapter, only species are listed whose presence has been confirmed in the northern and western mountains of Iranian via extensive field expeditions, examination of collections, literature review, as well as through personal communications with regional herpetologists. The classification adopted here is mostly conservative, though in some cases new, and to some extent, controversial names have been used.

In total 64 species of superfamilies Typhlopidea, Booidea and Colubroidea identified in the northern and western mountains of Iran. These identified species belong to seven families and 29 genera, as following: of family Typhlopidae and family Leptotyphlopidae, each family one genus and one species; of family Boidae, subfamily Erycinae, one genus and three species; of family Colubridae, two subfamilies, Colubrinae (with 14 genera and 39 species) and Natricinae (with one genus and two species); of family Lamprophiidae, subfamily

Psammophiinae, three genera and four species; of family Elapidae, two genera and two species; of family Viperidae, two subfamilies, Crotalinae (with one genus and one species) and Viperinae (with five genera and 11 species).

Superfamily Typhlopidea

Family Leptotyphlopidae

Genus *Leptotyphlops* Fitzinger, 1843

Leptotyphlops macrorhynchus (Jan, 1861)

Family Typhlopidae

Genus *Typhlops* Schneider, 1811

Typhlops vermicularis Merrem, 1820

Superfamily Booidea (Following Vidal & Hedges 2009)

Family Boidae

Subfamily Erycinae

Genus *Eryx* Daudin, 1803

Eryx (Eryx) elegans (Gray, 1849)

Eryx (Eryx) jaculus turcicus (Olivier, 1801)

Based on the examination of an extensive series of specimens from throughout its range, Tokar and Obst (1993) have shown that the Caucasus population of *E. jaculus* (that possibly occurs in northwestern Iran too), described by Eichwald (1831) as *E. familiaris* and recognized by Czarevsky (1916) as a subspecies of *E. jaculus*, is not distinguishable from southeast European-Turkish populations, heretofore referred to as *E. j. turcicus*. Thus, Tokar and Obst (1993) recognize only two subspecies, *E. j. jaculus* and *E. j. turcicus*.

Eryx (Eryx) tataricus (Lichtenstein, 1823)

Superfamily Colubroidea (Following Pyron *et al.*, 2011)

Family Colubridae

Subfamily Colubrinae

Genus *Boiga* Fitzinger, 1826

Boiga trigonatum melanocephala (Annandale, 1904)

Genus *Coluber* (s.l.)

Coluber (s.l.) *andreas* (Werner, 1917)

Genus *Coronella* Laurenti, 1768

Coronella austriaca (Laurenti, 1768)

Venchi and Sindaco (2006) state that the species is monotypic.

Genus *Dolichophis* Gistel, 1868

Dolichophis jugularis (Linnaeus, 1758)

Latifii (2000) reported *Dolichophis caspius* (Gmelin, 1779) for Iran but his identification needs verification.

Dolichophis schmidt (Nikolsky, 1909)

Genus *Eirenis* Jan, 1863

Nagy *et al.* (2003) propose a phylogeny based on molecular data, allocating the species to four subgenera: *Eirenis* Jan, 1863 (including *modestus* and *aurolineatus*), the new subgenus *Eoseirenis* (for *decemlineatus*), *Pseudocyclophis* Boettger, 1888 (for *persicus*) and *Pediophis* Fitzinger, 1843 (for all remaining taxa).

Latifii (2000) reported *Eirenis decemlineatus* (Duméril, Bibron, and Duméril, 1854) in Iran but his description shows he did a misidentification

Eirenis collaris (Ménétriés, 1832)

Eirenis coronella coronella (Schlegel, 1837)

Eirenis coronelloides (Jan, 1862)

Populations from SW Iran, S Iraq and NE Saudi Arabia cannot be assigned with certainty to any subspecies, although so far considered as belonging to *E. c. coronella* (Venchi and Sindaco, 2006).

Eirenis kermanensis Rajabizadeh, Schmidtler, Orlov and Soleimani, 2012

Eirenis medus (Chernov, 1940)

Eirenis modestus modestus (Martin, 1838)

Eirenis persicus (Anderson, 1872)

Eirenis punctatolineatus punctatolineatus (Boettger, 1892)

Eirenis punctatolineatus condone (Boulenger, 1920)

Eirenis rechingeri Eiselt, 1971

Eirenis walteri walteri (Boettger, 1888)

Eirenis walteri nigrofasciatus (Nikolsky, 1907)

Genus *Elaphe* Fitzinger, 1833

Utiger *et al.* (2002) split the western palaearctic species of *Elaphe* into three genera, reviving *Zamenis* Wagler for four species.

Elaphe dione dione (Pallas, 1773)

Elaphe sauromates (Pallas, 1814)

Genus *Hemorrhois* Boie, 1826

Hemorrhois nummifer (Reuss, 1834)

Hemorrhois ravergeri (Ménétriés, 1832)

Genus *Lycodon* Boie, 1826

Lycodon striatus bicolor (Nikolsky, 1903)

Genus *Lytorhynchus* Peters, 1862

Lytorhynchus ridgewayi Boulenger, 1887

Genus *Oligodon* Boie, 1826

Oligodon taeniolatus taeniolatus (Jerdon, 1853)

Genus *Platyiceps* Blyth, 1860

Platyiceps karelini karelini (Brandt, 1838)

Platyiceps najadum najadum (Eichwald, 1831)

P. n. atayevi (Tuniyev & Shammakov, 1993) occurs in Kopet Dag, Turkmenistan and probably adjoining Iran.

Platyiceps najadum schmidtleri (Schätti & McCarthy, 2001)

Platyiceps rhodorachis (Jan, 1865)

Platyiceps rhodorachis ladacensis (Anderson, 1871) has been considered a color pattern variation of *rhodorachis* ever since Anderson (1895) recognized that his species had already been described by Jan in 1865.

Platyiceps ventromaculatus ventromaculatus (Gray, 1834)

Genus *Rhynchocalamus* Günther, 1864

Rhynchocalamus melanocephalus (Nikolsky, 1899)

Genus *Spalerosophis* Jan, 1843

Baig and Masroor (2007) synonymized *Spalerosophis diadema schiraziana* (Jan, 1865) with *Spalerosophis diadema cliffordii* (Schlegel, 1837)

Spalerosophis diadema cliffordii (Schlegel, 1837)

Spalerosophis microlepis Jan, 1865

Genus *Telescopus* Wagler, 1830

Telescopus fallax ibericus (Eichwald, 1831)

Telescopus rhinopoma (Blanford, 1874)

Telescopus tessellatus martini (Schmidt, 1939)

Telescopus tessellatus tessellatus (Wall, 1908)

Genus *Zamenis* Wagler, 1830

Zamenis hohenackeri (Strauch, 1873)

In the past, two subspecies were recognized, the nominate and *taurica* (Werner, 1898); these were synonymized by Nilson & Andr  n (1984).

Zamenis longissimus (Laurenti, 1768)

Zamenis persica (Werner, 1913)

Subfamily Natricinae

Genus *Natrix* Laurenti, 1768

Natrix natrix natrix (Linnaeus, 1758)

Following the subspecific concept proposed by Thorpe (1975), only four subspecies are valid: *Natrix*, *cetti*, *corsa* and *helvetica*. The populations of the Eurasian mainland, North Africa and islands of the eastern Mediterranean Sea are divided into eastern, *N. n. natrix*, and western, *N. n. helvetica*,

subspecies (Guiking, *et al.*, 2006). However, subspecies have been described since, and Thorpe's review needs to be reevaluated.

Natrix tessellata tessellata (Laurenti, 1768)

Family Lamprophiidae Fitzinger, 1843

Subfamily Psammophiinae Günther, 1858

Genus *Malpolon* Fitzinger, 1826

Malpolon insignitus fuscus (Geoffroy Saint-Hilaire, 1827)

Carranza *et al.* (2006) confirmed the validity of the *Malpolon insignitus fuscus*.

Genus *Psammophis* Boie, 1826

Psammophis lineolatus (Brandt, 1838)

Psammophis schokari (Forsskål, 1775)

Genus *Rhagerhis* Peters, 1862

Rhagerhis moilensis (Reuss, 1834)

Brandstätter (1995) proposed to include this species in the monospecific genus *Scutophis*, on the basis of the micro-ornamentation of the scales.

Family Elapidae

Subfamily Elapinae

Genus *Naja* Laurenti, 1768

Naja oxiana (Eichwald, 1831)

Genus *Walterinnesia* Lataste, 1887

Walterinnesia morgani (Mocquard, 1905)

Family Viperidae

Subfamily Crotalinae

Genus *Gloydius* Hoge & Romano-Hoge, 1981

Gloydius halys caucasicus (Nikolsky, 1916)

Subfamily Viperinae

Genus *Echis* Merrem, 1820

Echis carinatus sochureki Stemmler, 1969

Genus *Macrovipera* Reuss, 1927

Macrovipera lebetina cernovi (Chikin et Szczerbak, 1992)

Macrovipera lebetina obtusa (Dwigubsky, 1832)

According to Venchi and Sindaco (2006), the ssp. *obtusa* Dwigubskij, 1832 includes *euphratica* Martin, 1838, *turanica* Černov in Terent'ev & Černov, 1940 and *cernovi* Chikin & Ščerbak, 1992.

Genus *Montivipera* Nilson, Tuniyev, Andrén, Orlov, Joger and Herrmann

Montivipera latifii (Mertens, Darevsky and Klemmer, 1967)

Montivipera raddei raddei (Boettger, 1890)

Montivipera raddei albicornuta (Nilson and Andrén, 1985)

For a taxonomic revision of the Genus *Montivipera* in Iran, see chapter four.

Genus *Pseudocerastes* Boulenger, 1896

Pseudocerastes persicus (Duméril, Bibron, and Duméril, 1854)

Pseudocerastes fieldi K. Schmidt, 1930

Pseudocerastes fieldi reported by Gholamifard et al. (2010) from fars prov. Southern Zagros

Pseudocerastes urarachnoides Bostanchi, S. Anderson, Kami, and Papenfuss, 2006

Genus *Vipera* Laurenti, 1768

Joger et al. (1992) identify five “evolutionary groups (species)” within the *V. kaznakovi*-*ursinii* complex: 1) *ursinii* including all European subspecies, 2) *renardi* including *eriwanensis* and Central Asian “*ursinii*”, 3) *anatolica*, 4) *darevskii*, 5) *kaznakovi* with *dinniki* and *orlovi*. Ferchaud et al. (2012) observed close relationship between the Caucasus and Iranian species (*Vipera ursinii* and *Vipera ebneri*) with the *Vipera renardi*.

Vipera eriwanensis eriwanensis (Reuss, 1933)

Vipera eriwanensis ebneri Knoepffler and Sochurek, 1955

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Abstract

Snakes are the second most speciose group of living reptiles. Colubroidea, with more than 2500 species, is one of the most conspicuous radiations of terrestrial vertebrates. Recent studies show that globally 12% of the snake species are estimated to be threatened with extinction. This estimation increases when including the cryptic species (discrete species that are difficult, or sometimes impossible, to distinguish morphologically and thus have been incorrectly classified as a single taxon) too. It is possible that some of the snakes biodiversity may get extinct even before a proper identification. In the developing countries like Iran this situation is even more serious. The country of Iran has the greatest faunal and floral diversity of the southern and arid parts of the Palearctic realm, including a considerable diversity in the snakes. But there is insufficient data on the biodiversity of snakes in Iran and limited data about cryptic species of snakes in the country. In this PhD study, I have aimed to investigate the taxonomy of selected species of Iranian snakes, inhabiting the northern and western mountains of Iran. Relevant taxa were detected based on a review performed on the available taxonomic data on snakes of Iran. Snake taxa with a wide distribution range that reflect complexity of adaptations to different ecosystems or have great matter of taxonomic confusion, including the *Eirenis medus* species complex, the *Eirenis persicus* species complex, the *Eirenis punctatolineatus*, the *Montivipera raddei* species complex, and *Natrix tessellata*, have been selected for this study.

Samplings were done in the northern and western mountains of Iran between 2008 and 2011. Patterns of morphological variation and its relevance for taxonomical interpretations of the *Montivipera raddei* species complex, as well as the taxonomy of *Natrix tessellata*, *Eirenis punctatolineatus* and *Eirenis medus* species complex, were investigated using a principal component analysis (PCA) and a multivariate analysis of variance (MANOVA) on metric and meristic data of the specimens. Taxonomy of the *Eirenis persicus* species complex was studied using both traditional biometry and geometric morphometry on landmark data of the dorsal head scales. Two mitochondrial genes, 16S rRNA and cytochrome *b*, and one nuclear gene, *c-mos*, were sequenced and then a concatenated data set of these was analyzed to generate a Bayesian inference of phylogeny.

The morphological analysis on the *Montivipera raddei* species complex revealed that a couple of specimens of the genus *Montivipera*, originating from the central Zagros mountain range,

is representing an unknown species. This species, named *Montivipera kuhrangica* Rajabizadeh, Nilson and Kami, 2011, has a higher count of head scales than other closely related species, including higher a number of intercanthal, supralabial scales and scales between the last supralabials. The field data indicate that in the *Montivipera raddei* species complex, there is a correlation between the dorsal color pattern variation and the environmental factors of the habitat, including vegetation and substrate color. In order to study geographic variation of *Natrix tessellata* in Iran, a total of 66 specimens, including 39 males and 27 females, were examined. The analysis on both sexes separately revealed that the males from the Fars province (southern Zagros) are significantly different from all other dice snakes in Iran, in the tail length, head length, head width and number of ventral scales. Further studies are needed to resolve the taxonomic problem of the southern Zagros populations of *Natrix tessellata*. The analysis on 23 males and 21 females of *Eirenis punctatolineatus* indicates that the southern Zagros populations differ significantly from the rest of the populations in tail length and number of subcaudal scales, number of prefrontal scales, number of scales bordering parietals and shape of the dorsal color pattern in both sexes. A subspecific subdivision is suggested for the northern and southern populations. As a new combination, *Eirenis punctatolineatus condone* (Boulenger, 1920), is proposed for the southern populations. Analysis on the three populations of *Eirenis medus* species complex from Turkmenistan and northern and western mountains of Iran, revealed a couple of specimens from Kerman province in southern Zagros that differs from known populations of *Eirenis medus* in their morphometric and meristic characters. Compared with *Eirenis medus*, this new species, named *Eirenis kermanensis* Rajabizadeh, Schmidtler, Orlov and Soleimani, 2012, has a higher number of subcaudals and a higher ratio of tail length over total length. Also in *Eirenis kermanensis*, the loreal scale is deeper than long, while in *Eirenis medus* the loreal is longer than deep. In studying the taxonomy of the *Eirenis persicus* species complex, the biometric data from 23 males and 17 females revealed that the populations of *Eirenis persicus* in the western mountains of Iran and Turkey are significantly different from the populations in eastern Iran, Turkmenistan and Pakistan. This significant divergence was confirmed by geometric morphometric and molecular analyses too. Both molecular and morphological analyses showed that specimens of the *Eirenis persicus* species complex that belong to the *nigrofasciatus* morph, are closely related to the eastern populations of the *Eirenis persicus* species complex. Within-group PCA revealed that *Eirenis persicus* populations

of Iran and Turkey are morphologically different from each other at a subspecific level. This result was confirmed by molecular data as well.

Studies on the genus *Eirenis* revealed the occurrence of cryptic biodiversity in these snakes. During the warming periods of the Pleistocene climatic oscillations, the suitable habitat of mountain dwelling snakes shifted to higher altitudes in the mountain. Subsequently, climatic cooling shifted the range of these habitats back to lower altitudes. These altitude shift of the habitat during the Pleistocene climatic oscillations induced the isolation and speciation in the examined snake species throughout the northern and western mountains of Iran. The present study on the selected species of the genera *Eirenis*, *Natrix* and *Montivipera* confirmed that the current knowledge on Iranian snake taxonomy is limited, and undescribed species remain to be discovered. This limited knowledge is to be understood due to the substantial snake biodiversity in Iran (related to its diversity of ecosystems) and lack of in-depth taxonomic studies on snake biodiversity. As such, although most of the results from this study are new related to our understanding of snake biodiversity in Iran, they still cover only a few species of this Iranian diversity, of which a lot remains to be properly assessed.

Samenvatting

Onder de recente reptielen, vormen slangen één van de meest diverse groepen. Alleen binnen de Colubroidea zijn meer dan 2500 species gekend. Recent onderzoek toont aan dat globaal 12% van de soorten slangen beschouwd worden als bedreigd met uitsterven. Het feit dat veel soorten een cryptische diversiteit vertonen, waarbij het dikwijls moeilijk tot onmogelijk is om natuurlijke groepen te onderscheiden, impliceert dat dit een onderschatting zal zijn. Dit is vooral van toepassing bij minder ontwikkelde landen die een zeer grote diversiteit kennen, zoals het geval is voor Iran, met zijn zuidelijke en droge gebieden van het Palearctisch gebied. Desondanks zijn grote diversiteit, is er heel weinig gekend over de cryptische diversiteit binnen de slangen in Iran. De doelstelling van dit onderzoek is dan ook om daar een bijdrage aan te leveren, waarbij de taxonomie van enkele taxa kritisch wordt geëvalueerd. De aandacht is vooral gegaan naar soorten die voorkomen in de noordelijke en westelijke bergketens van Iran, en die onderwerp zijn van heel wat taxonomische onduidelijkheden: *Eirenis medus* species complex, *Eirenis persicus* species complex, *Eirenis punctatolineatus*, *Montivipera raddei* species complex en *Natrix tessellata*.

Veldwerk in deze noordelijke en westelijke bergketens van Iran werd uitgevoerd tussen 2008 en 2011. Biometrische, morfometrische en kwalitatieve kenmerken werden verzameld en onderworpen aan principale componenten analyses (PCA), gekoppeld aan multivariate tests om na te gaan in welke mate geografische populaties van elkaar kunnen worden onderscheiden. Daarenboven werden twee mitochondriale genen (16S rRNA en cytochrome *b*) en één nucleair gen (*c-mos*) gesequeneerd en verwantschappen tussen individuen en populaties geanalyseerd via Bayesian inference.

De morfologische studie op de *Montivipera raddei* species complex toonde aan dat een aantal specimens afkomstig uit de centrale Zagros keten als een nieuwe soort dienen te worden beschouwd, *i.e.* *Montivipera kuhrangica* Rajabizadeh, Nilson and Kami, 2011. Dit steunt op de aanwezigheid van grotere aantallen schubben op verschillende plaatsen op de kop, vergeleken met verwante soorten binnen dit geslacht. Observaties in het veld toonden ook aan dat binnen de *Montivipera raddei* groep, er een relatie bestaat tussen het dorsaal kleurpatroon en kenmerken van het habitat (zoals vegetatie en kleur van de ondergrond). Voor de analyse van geografische variatie binnen *Natrix tessellata* in Iran, werden 66 specimens (39 mannetjes en 27 wijfjes) bestudeerd. Een afzonderlijke analyse per geslacht

toonde aan dat mannetjes afkomstig van de Fars provincie (zuidelijk Zagros) significant verschillend zijn van alle andere individuen binnen deze soort (o.a. op basis van de lengte van de staart, de kop en bepaalde schubben). Verder onderzoek dient nog te bevestigen of dit verdere taxonomische implicaties heeft of niet. Bij *Eirenis punctatolineatus* bleek ook dat de populatie van de zuidelijke Zagros significant verschillend is van de andere populaties op basis van de lengte van de staart en aantallen schubben op verschillende plaatsen op het lichaam. Op basis daarvan kan een onderverdeling op ondersoort-niveau worden voorgesteld voor de zuidelijke en de noordelijke populaties. Voor de zuidelijke populatie wordt de naam *Eirenis punctatolineatus condone* (Boulenger, 1920) voorgesteld. De analyse van de drie populaties binnen de *Eirenis medus* species complex (afkomstig uit Turkmenistan en de noordelijke en westelijke bergketens in Iran) toonde eveneens aan dat specimens konden worden onderscheiden die significant verschillend waren (o.a. op basis van unieke kenmerken in de vorm en aantallen schubben). Deze zijn afkomstig van de Kerman provincie (zuid Iran), en worden als een nieuwe soort beschouwd: *Eirenis kermanensis* Rajabizadeh, Schmidtler, Orlov and Soleimani, 2012. Daarenboven kon worden aangetoond dat binnen de *Eirenis persicus* species complex, de populatie uit de westelijke bergketens en Turkije significant verschillend zijn van de populaties van oostelijk Iran, Turkmenistan en Pakistan. Dit verschil uitte zich zowel op basis van de geometrisch morfometrische data als de moleculaire data, evenals dat hieruit kon worden afgeleid dat de specimens binnen de *Eirenis persicus* species complex met een *nigrofasciatus* kleurpatroon, nauwer verwant zijn aan die van de oostelijke populaties binnen de *Eirenis persicus* species complex. De analyses toonden verder ook aan dat de *Eirenis persicus* populaties uit Iran en Turkey ook onderling van elkaar verschillen, en dat dit verschil toe moet laten om beide als ondersoorten te erkennen (mede doordat dit ook moleculair werd ondersteund).

Dit onderzoek toont dus aan dat er wel degelijk cryptische diversiteit aanwezig is binnen het geslacht *Eirenis*. De waargenomen isolatie van de zuidelijke Zagros populaties binnen zowel *Natrix tessellata* als *Eirenis punctatolineatus* kan verklaard worden als gevolg van Pleistocene klimatologische oscillaties. Dit onderzoek op de geselecteerde soorten binnen de genera *Eirenis*, *Natrix* en *Montivipera* bevestigen dat de huidige kennis van de diversiteit van de slangen uit Iran beperkt is, en dat verder onderzoek moet aantonen welke ongekennde diversiteit bestaat.

Appendix 1: List of the examined specimens of *Montivipera raddei* species complex (N=67)

Montivipera latifii; 1: personal collection of Rajabizadeh, L01 – 5, A01, A02, A03 – A05 (Lar national park, Tehran prov./♂♂♂♂♂/2006-2007), 2: personal collection of Rajabizadeh, A01 - 3, A05 (Afjeh, Tehran prov./♀♂♀♀), 3: RI 18792, 12918, 2445, 1341, 13007, 23362, 24922, 2764, 1432, 13106, 9255, 9283, 24974 (central Alborz, Tegan and Mazandaran prov./♂♂♂♂♀♀♀♀♂♂♂)

Montivipera raddei albicornuta; 4: personal collection of Rajabizadeh, KH02 - 5 (Khanchay, Zanjan prov./♂♀♂♂♀ /2006-2007), 5: personal collection of N. Stümpel, Nik06 – 9 (Khanchay, Zanjan prov./♀♂♀♂/2005).

Montivipera raddei raddei; 6: MTKD D016868, D026624, D08572, D019783, D012347, D011864, D016867, D012514, D012515, D026623, D08573, D011179 (Armenia republic/♂♂♂♂♂♀♀♀♀♀♀), 7: MTKD D029250 (eastern Turkey/♀), 8: personal collection of Rajabizadeh, K01 – 3 (Chelcheshmeh mountain, Kordestan prov. Iran/♂♀♂), 9: personal collection of Rajabizadeh, T01 – 3 (Tekab mountains, Zanjan prov. Iran/♀♀♀), 10: personal collection of Rajabizadeh, M01 – 2 (around Maku, western Azarbaijan, Iran/♂♀), 11: MMTT? (around Maku, western Azarbaijan, Iran/♂/2007), 12: personal collection of Rajabizadeh, S01 – 2 (Sahand and Sabalan mountain, eastern Azarbaijan, Iran/♀♂), 13: personal collection of N. Stümpel, Nik03 -5, Nik12 – 13 (eastern Turkey/♀♂♀♀♂/2005), 14: personal collection of Rajabizadeh, Gh01 (Ghotur village, western Azarbaijan, Iran/♂/2006).

Montivipera kuhrangica; 15: ZMGU 2203 (Tulip valley (Darreye Lale or Dashte Lale) (32°36 N 50°11 E), 8 km north east of Chelgerd village, Kuhrang region, Chaharmahal and Bakhtiari province, Iran/♂), 16: ICSTZM7H1137 (Kuhrang region, Chaharmahal and Bakhtiari province, Iran/♂/2012).

Appendix 2: List of the examined specimens of *Natrix tessellata* species group

(N=66). Specimens are collected in the last 10 years.

1: ZUTC.Rep1370, Rep1371, Rep1374, Rep1375, Rep1361, Rep1362, Rep1363, Rep1365, Rep1369, Rep1373, Rep1376, Rep1364, Rep1366, Rep1368 (Anzali lagoon, Gilan prov./♂♂♂♂♂♂♀♀♀♀♀), 2: CBSU 4353 (Arasanjan, Fars prov./♂), 3: CBSU 4787, R069, R070, 4788 (Marvdasht, Fars prov./♂♂♂), 4: CBSU C882 (Dashte arjan, Fars prov./♂), 5: CBSU S1-3 (Beiza, Fars prov./♀♂♂), 6: CBSU C884 (Abadeh, Fars prov./♀), 7: RUZM.2, 10, 9, 6, 12, 14, 15, 8, 3, 5 (around Kermanshah city, Kermanshah prov./♂♂♂♂♂♂♀♀♀♀♀), 8: RUZM.37, 41 (Shahrekord, Lorestan prov./♀♀), 9: RUZM.5 (Khansar, Isfahan prov./♀), 10: CBSU C881 (Kazerun, Fars prov./♀), 11: CBSU C884 (Abadeh, Fars prov./♀), 12: CBSU 5024, 5392 (Shiraz, Fars prov./♂♂), 13: ZMGU 1194 (Alagol, Golestan prov./♂), 14: ZMGU 481, 1004 (Mazandaran prov./♂♂), 15: ZMGU 1914 (Sabzevar, Khorasan Razavi prov./♂), 16: ZMGU 148 (Ghuchan, Northern Khorasan prov./♂), 17: ZMGU 1001 (Mianeh, Eastern Azarbaijan prov./♂), 18: RUZM.11 (Ajabshir, Western Azarbaijan prov./♂), 19: CBSU B688 (Tabriz, Eastern Azarbaijan prov./♂), 20: ICSTZM7H1070 (Lar valley, Tehran Prov./♂), 21: CBSU B688 (Barmshur, fars prov./♂), 22: DEZC.H122 9Varzaneh, Isfahan prov./♂), 23: DEZC.H123 9Afus, Isfahan prov./♂), 24: ZMGU 46, 30 (Dezful, Khuzestan prov./♂♀), 25: ZMGU 1568, 34, 995, 1268, 488 (Mazandaran prov./♀♀♀♀♀), 26: ZMGU 1000, 1002 (Mianeh, Eastern Azarbaijan prov./♀♀), 27: ZMGU 445 (around Ahvaz city, Khuzestan prov./♀).

Appendix 3: List of the examined specimens of *Eirenis puntatolineatus* (N=44)

1: ZSM 1768/2005 and ZSM 2782/2005 (Develi village, 60Km W. Malatya, Malatya prov. /38°13'N, 37°39'E/ ≈1400m/ ♂♂), 2: ZSM 3221/2005 (Aydinkavak, E Kagizman, Kars prov. /40°09'N, 43°18'E/ ≈1400m/ ♀), 3: ZSM 90/1918/1 – 5 (Mukus village, south of Catak, Siirt prov. /38°00'N, 43°03'E/ ♂♂♂♀♀), ZSM 285/1988 (Edremis, Van prov.) /38°12'N, 42°36'E/ / ♀), ZSM 3506/2005 (Akdamar Island, Lake Van, Van prov. /38°20'N, 43°04'E/ ≈1700m/ ♂), 4: ZSM 285/1988 (Zap-Tal (valley), 24Km E Hakkari, Hakkari prov. /37°34'N, 44°02'E/ ♀), 5: ZMUM1-6 (Yerevan, Armenia /♂♂♂♀♀♀), 6: ICSTZM7H1033-34 (Varankesh village, S. Bozghush mountain, E. Azarbaijan prov. /37°40'N, 47°33'E/ 1500m/ ♂♂), ZMGU1006, (around Mianeh, E. Azarbaijan prov. /37°26'N, 47°43'E/ ♂), 7: ZSM 1178/2006 (around Qazvin, Qazvin prov. /36°17'N, 50°01'E/♂), 8: (around Damghan /36°07'N, 54°20'E/), 9: MMTT1091 (22Km S. of Quchan-Mashhad junction, road to Soltanabad village, Khorasan Razavi prov. /36°43'N, 59°04'E/ ≈1200m/♂), 10: RUZM-CEX7.1-8 & RUZM-SN0155 (Kermanshah prov./ ♀♀♂♀♂♀♂♂), 11: ICSTZM7H1101 (around Poldokhtar, Lorestan prov. /33°13'N, 47°42'E/ ♂), 12: CBSU4452 (S Isfahan prov. /♀), 13: CBSU8048 (Sisakht, Yasuj prov. /30°50'N, 51°29'E/ >2300m/♀), 14: CBSU4402 & CBSUB696 (Nurabad, Fars prov. /30°05'N, 51°36'E/ ♂♀), 15: CBSU5304, CBSU8027, CBSU8053, CBSU8006, CBSU** & CBSU ** (around Shiraz including Shiraz, Beiza & Dehsheikh / 29°39'N, 52°28'E/ >1500m/ ♀♂♀♂♂♀), 16: CBSU5396 (Khafr village, Fars prov. /28°58'N, 53°12'E/♀), 17: ZMGU84-85 (Jahrom Airport, Fars prov. /28°30'N, 53°33'E/ 1000m/ ♀♀), 18: CBSUB695 (Estahban, Fars prov. /29°06'N, 54°01'E/♂), 19: CBSU4521 (Darab, Fars prov. /28°44'N, 54°33'E/♂), 20: CBSU4459 (Larestan, Fars prov. /27°39'N, 54°17'E/♂), 21: ICSTZM7H1035 (Sarduieh, Kerman prov. /29°15'N, 57°16'E/♂), 22: DHZC.H 109 & ICSTZM7H1012 (Geno protected area, Hormozgan /27°22'N, 56°10'E/900/ ♂♀), 23: ICSTZM7H1004-8 (around Bam, Kerman prov. /29°08'N, 58°18'E/ 1100m/ ♀♀♂♀♂♂), 24: MMTT1072 (80 km south of Aghajari, on road from Behbahan to Genaveh, Bushehr Prov. / 30°08'N, 50°20'E/ about 200m/ ♀).

Appendix 4: List of the examined specimens of *Eirenis persicus* species complex

(N=40)

***Persicus* morph, western Iranian plateau;** 1: ZSM 1711/2005 (Halfeti'nin 4 km Kuzeyi, Şanlıurfa, Turkey/♀), 2: ZSM 3242/2005 (Viranşehir'in 22 km batısı, Şanlıurfa, Turkey /♂), 3: ZSM 1467/2005, ZSM 1469/2005 (Doğanşehir, Malatya, Turkey /♀♂), 4: ZSM 1801/2005 (Kangal'in 49 km Güneydoğusu, Malatya, Turkey /♀), 5: ZSM 569/2002 (Viranşehir'in 22 km batısı, Şanlıurfa, Turkey /♂), 6: ZSM 565/2002 (Halfeti'nin 4 km Kuzeyi, Şanlıurfa, Turkey /♂), 7: ZSM 2701-3/2005 (Sorkh-e-Dize, 120 km W Kermanshah, İran/♀♀♀), 8: ZDEU 94/2005 (Şanlıurfa-Hilvan arası 32. Km, Şanlıurfa, Turkey /♂), 9: ZDEU 136/2005, 136/2005 (Diyarbakır-Siverek Arası 48. Km/Diyarbakır, Turkey/♂♀), 10: CBSU R086, CBSUB632, CBSU 5387, CBSU 4536, CBSU 4538, CBSU R088, CBSU 4525 (Fars prov., İran/♂♂♂♂), 11: ICSTZM 7H1107-8 (Dashte Arjan, fars prov., İran/ ♂♀/2012), 12: ICSTZM7H1120 (Hanna dam, Semirom, Isfahan prov, İran/ ♀/2012),

***nigrofasciatus* morph;** 13: ICSTZM 7H1113 (Fars prov, İran/♀), 14: RUZMCPPO.16-17 (Ilam prov, İran/♀♂), 15: CBSU 4538, CBSU R088, CBSU 4525 (Fars prov., İran/♂♀♂), 16: ICSTZM 7H1009 (Khabr NP, Kerman prov, İran/ ♂/2009), 17: ICSTZM 7H1121 (Vahdati, Dezfül, Khuzestan prov, İran/♂/2012),

***novum* morph;** 18: CBSU 8047 (Mishi spring, Sisakht, Yasuj prov, İran/ ♂), 19: CAS 247041 (At tamim, Iraq/♂)

***persicus* and *walteri* morph, eastern Iranian plateau;** 20: ICSTZM 7H1135 (Dehbakri, Kerman prov, İran/♂/2012), 21: ICSTZM 7H1084 (Golestan prov, İran/♂), ZMMU3252-5 (Turkmenistan/♂♀♀♀♂♂), 22: NHM 1900.7.10.1-2, 23.10.13.40-2 (north western Pakistan/ ♀♂♂♂♂)

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2- **Book chapter with peer review**

Rajabizadeh, M., Javanmardi, S., Rastegar-Pouyani, N., Karamiani, R., Yusefi, M., Salehi, H., Joger, U., Mebert, K., Esmaeili, H. R., Parsa, H., Kami, H. G. & Rastegar-Pouyani, E. (2011) - Geographic Variation, Distribution and Habitat of *Natrix tessellata* (Laurenti, 1768) (Serpentes: Colubridae) in Iran. Mertensiella, Vol. 18. The Dice Snake, *Natrix tessellata*: Biology, Distribution and Conservation of a Palearctic Species. Edited by Konrad Mebert, on behalf of the Deutsche Gesellschaft für Herpetologie und Terrarienkunde. ISSN 0934-6643 ISBN 978-3-9812565-4-3. Rheinbach, Germany. pp: 414-429